

DYNAMIC PHOTOSYNTHETIC RESPONSE OF SOYBEANS:
MODEL DEVELOPMENT AND ELEVATED CO₂ EXPERIMENTS

By

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Abstract of Dissertation Presented to the Graduate
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DYNAMIC PHOTOSYNTHETIC RESPONSE OF SOYBEANS:
MODEL DEVELOPMENT AND ELEVATED CO₂ EXPERIMENTS

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A biochemical photosynthesis model has been developed and experiments at the whole plant level have been conducted in a holistic investigation of photosynthetic rate controls. The model was based on known biochemical pathways and emphasized the roles of starch, sucrose and inorganic phosphate in the feedback control of CO₂ fixation. The experimental work was designed to investigate short term rate response to a sudden change in CO₂ concentration and long term physical adaptation to distinctive environmental CO₂ levels. In the experimental procedure different source-sink balances were established for each treatment and plant response was measured. In the model the mechanisms for source-sink feedback control of the biochemical CO₂ fixation process were developed. The overall purpose of the work has been to demonstrate and clarify the role played by source-sink relations in the feedback regulation of photosynthesis.

Comparison of model predictions with measurements from the literature have shown the model's individual response equations to behave well.

Overall the model demonstrated the central role of inorganic phosphate as a regulator of the Calvin cycle via its affect on the ATP/ADP ratio and as a regulator, partitioning CO₂ between starch and sucrose synthesis. In turn, the concentration of inorganic phosphate was modelled to depend inversely on cytoplasmic sucrose level which was modelled to depend on export. By functionally linking the export of sucrose from the cytoplasm to sink demand, the model qualitatively described the photosynthetic inhibition and enhancement observed in a variety of scenarios.

The experimental results showed soybeans (Glycine max c.v. Bragg) to respond to elevated CO₂ levels by increasing net photosynthesis and respiration in the short term and increasing leaf area and biomass in the long term. Close analysis of photosynthetic light response found morning enhancement and/or afternoon inhibition of CO₂ fixation in plants exposed to high [CO₂]. In the canopy switched to high CO₂, the magnitude of afternoon inhibition was suppressed by rapidly enhanced sink strength. Reduced CO₂ fixation corresponded to higher levels of soluble carbohydrates in the leaves. Taken together these results supported the proposed interdependence between leaf sucrose levels and photosynthesis.

The experimental work showed the association between source-sink balancing and photosynthetic rate while the biochemical model demonstrated a linkage mechanism. The differences in detail of the biochemical and whole plant levels prevented direct quantitative comparisons between model and experimental results. Nevertheless the experimental and model results were qualitatively consistent and this work represented a necessary effort at a holistic explanation of photosynthetic rate regulation.

INTRODUCTION

All living systems in the biosphere are based directly or indirectly on sunlight, carbon dioxide and water. Photosynthesis is the process common to green plants that combine these elements into energy-rich carbohydrates. Within plant systems the carbohydrates produced by photosynthesis have two functions: first, as substrate for plant tissue synthesis and second, as energy for the synthesis reactions. As some of the carbohydrate building blocks are combined with nitrogen, sulfur and other elements into plant tissue, others are respired releasing required reaction energy.

Photosynthesis occurs in plant leaves while tissue synthesis and respiration use carbohydrates throughout the plant structure. Carbohydrates are translocated from the production source in the leaves to consumption sinks through the phloem which directly connects the photosynthetic leaf material to active reaction sites.

More specifically, the chain of events that provides carbohydrates for plant growth begins in microscopic organelles called chloroplasts which are suspended in the cytoplasm of photosynthetic cells. Carbon dioxide diffuses from the atmosphere to the chloroplasts where it is chemically reduced in a process called the Calvin cycle, driven with energy supplied by light. The assimilated CO_2 is either converted to sucrose in the cytoplasm, or to starch in the chloroplast, both of which are carbohydrates. A schematic representation of this system is given

in Figure (1). The translocation system begins with the export of a three carbon phosphorylated compound (dihydroxyacetone phosphate, DHAP) from the chloroplast to the cytoplasm, where it is converted to sucrose, which moves into the phloem for distribution throughout the plant. The balance between CO_2 fixation in the chloroplast and carbohydrate export from the cytoplasm is important to photosynthetic feedback control, but is poorly understood.

The balance between carbohydrate sources and sinks can be disrupted by changing environmental conditions. When this happens, photosynthesizing cells must adjust to the new circumstances. If photosynthetic rate is increased or if transport to the phloem is decreased then excessive amounts of carbohydrates may accumulate in the photosynthetic cells. When this occurs starch is reversibly formed in the chloroplasts, storing fixed carbon until it can be mobilized and shipped to the cytoplasm for conversion to sucrose and export to the rest of the plant. If starch accumulation continues past a certain point, cells can be physically damaged [Noble and Craig, 1973]. It is postulated that before damage occurs, the plant, under normal conditions, adapts by either decreasing its rate of photosynthetic supply of carbohydrates, or by increasing its rate of sucrose translocation and utilization.

Changes in the rate of starch accumulation have been observed in response to many situations. For instance, when plants have been switched from atmospheres with ambient levels of CO_2 to enriched CO_2 conditions, starch accumulation and photosynthesis increased dramatically but while starch levels continued to rise after several hours, photosynthetic rates declined [Mauney et al., 1979]. In another example, plants acclimated to warm nights were found to have increased

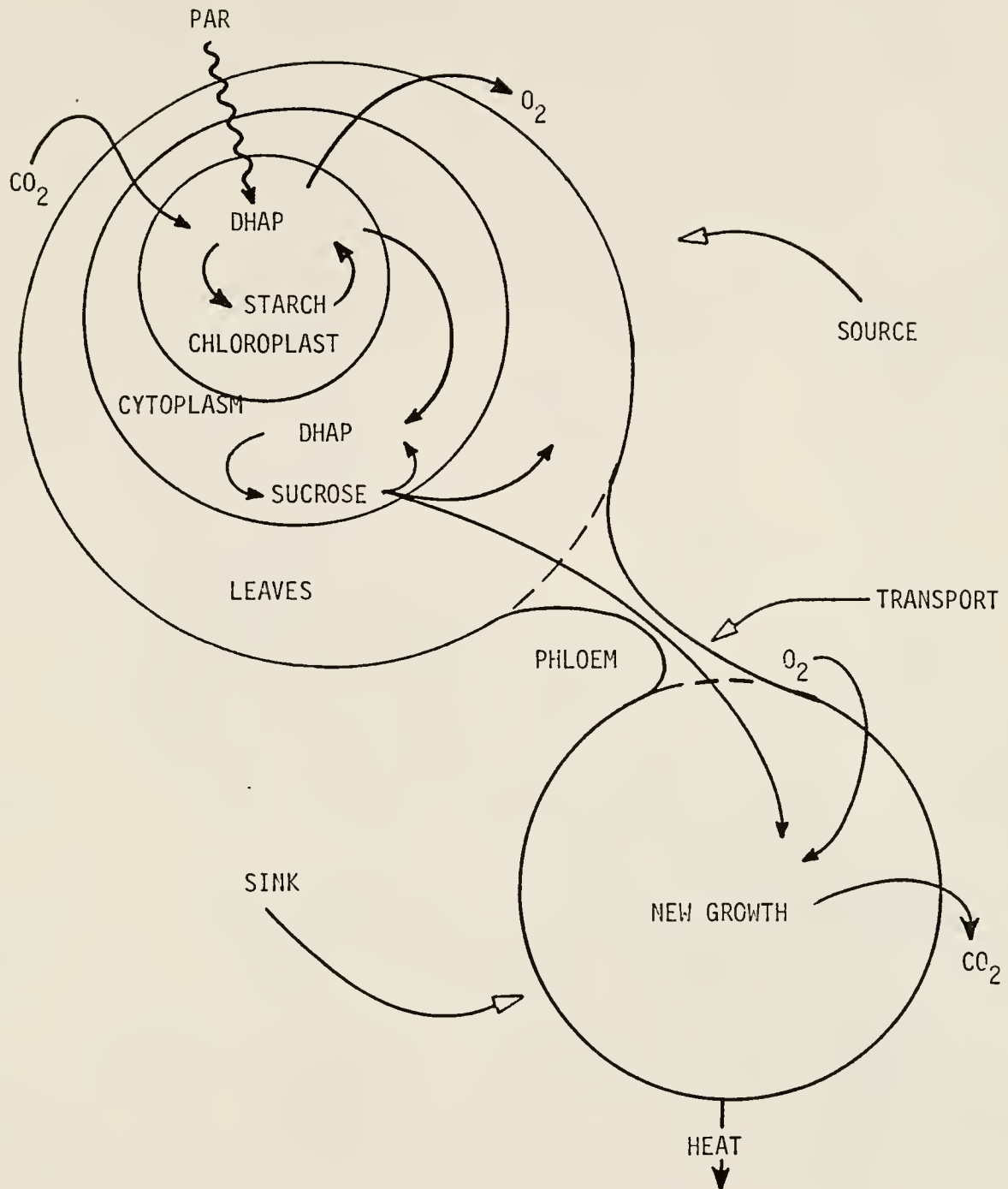


Figure 1. Photosynthesis and source-sink balance schematic. PAR is photosynthetically active radiation. All abbreviations are defined in Appendix 1. Terms are defined in Appendix 2.

starch concentrations and decreased photosynthetic rates following exposure to low night time temperatures.

In the case of high $[CO_2]$, the plant's mature leaves exported more carbohydrates than they had in low $[CO_2]$, but not enough to balance the increased supply. Consequently, the excess was stored as starch. In the case of low night temperatures, carbohydrates normally used in night time growth and maintenance processes were not used due to temperature dependent reductions in process rates. On the following day reduced sink demand caused carbohydrate levels in the photosynthetic cells to increase.

Observations such as these are often explained conceptually in terms of imbalances in the source-sink relationship. Whether the carbohydrate levels increase due to high CO_2 fixation or reduced sucrose export, the system balances itself by reducing photosynthesis. On the other hand, when sink strength is increased and carbohydrate levels in the photosynthetic cells decline, maximum photosynthetic rates are observed to increase [Thorne and Koller, 1974]. Source-sink balancing is superficially a straightforward and satisfying explanation for alterations in photosynthetic response. Unfortunately, the details of the proposed source-sink mechanism are not well understood.

The photosynthetic system consists of complex and interrelated processes all of which serve to control the overall CO_2 fixation rate. In simple terms, every plant's physical and chemical environment is continuously changing. In turn, compound levels and process rates which determine photosynthetic rate within the plant are also changing. In this sense, plant photosynthesis is dynamic.

Macroscale variations in photosynthetic capacity are a function of nutrient availability, water supply, temperature, sunlight, O_2 concentration and CO_2 concentration. At the cellular level, photosynthetic capacity is a function of enzyme availability, enzyme activity and substrate concentrations. All of these factors combine to establish individual reaction rates, the products of which are substrates for subsequent reactions. The integrated sum of the various reactions control particular process rates. Finally, whole plant parameters and biochemical processes are functionally coupled by transport systems some of which are passive and some of which are active. In both cases concentration gradients and transport resistances are determining factors in the mass transport rates of substances from the environment to the biochemical reaction sites of photosynthesis. The sum of these interactions determines the instantaneous rate of CO_2 fixation.

This brief holistic description of photosynthesis emphasizes that response should be considered at both the whole plant and biochemical levels of plant organization. Modellers began trying to relate external environmental conditions to photosynthesis as a biochemical process thirty years ago [Rabinowitch, 1951]. Since then, a steady stream of increasingly biochemical models has been developed [Chartier, 1970; Charles-Edwards and Ludwig, 1974] leading to the biochemical models proposed by Peisker [1974] and more recently by Farquahr et al. [1980].

One of the main criteria which these modellers have set is the adequate simulation of various photosynthetic light response curves. Usually, results from experimental work on photosynthesis are presented graphically in photosynthetic light response curves which plot carbon dioxide exchange rate (CER) against light level. Because of the very

strong dependence of CO_2 assimilation on light level, the graphical relationship between them has long been considered a fundamental measure of response, whether research is on whole plant canopies or reconstituted chloroplasts.

The fundamental importance of the photosynthetic light response curve (PLRC) is further enhanced by the similar shape of most plots generated for a wide variety of plants, which implies very similar underlying mechanisms. Several distinct functional equations have been proposed which generate curves of the appropriate shape [Thornley, 1976]. However, the most commonly derived equations are variations of the rectangular hyperbola. This particular equation is scientifically satisfying because of its theoretical basis in enzyme kinetics, where it is called a Michaelis-Menten response curve. Because of the cyclical enzymatic pathways which photosynthesis follows, it seems quite natural that the rate of CO_2 uptake should have a Michaelis-Menten form. The goal for modellers has been to relate light via an assumed biochemical pathway to CO_2 uptake in such a way that external parameters could be used to represent the variations in photosynthesis in response to light.

Photosynthesis models such as those described above are currently used in conjunction with other crop system models to predict growth rates and yield. These models concentrate on the uptake of CO_2 in response to light level while ignoring how or why fixed carbon is partitioned between starch and sucrose. As a result, they work well under normal conditions but are generally inadequate for describing response to unusual circumstances.

It is hypothesized that partitioning between stored chloroplastic starch and cytoplasmic sucrose is central to the feedback control of

photosynthesis. As noted in the examples of low night temperatures and increased CO_2 levels, starch accumulation is often reported in association with reduced photosynthetic response. To model this relationship, starch and sucrose cannot be simply divided into unrelated pools.

Synthesis of starch and sucrose must respond functionally to mechanisms which prescribe how fixed carbon is to be partitioned. One of the main goals of this research has been to develop a model based on such mechanisms.

To accomplish this goal a detailed photosynthesis model has been developed, based on five specific biochemical pathways:

1. the light reactions
2. the Calvin cycle,
3. the chloroplastic starch cycle,
4. the cytoplasmic sucrose pathway, and
5. the phosphate translocator system.

The purpose of the developed model is to consider the hypothesized roles that starch and sucrose play in the feedback control of CO_2 fixation. This is done by identifying the possible interactions among the five pathways that could limit photosynthesis and control partitioning. From this perspective it becomes possible to investigate the complex relationship between biochemical dynamics and the ambient environment. Such a detailed model can also be used to suggest how the photosynthetic system might respond in the long term to different prevailing environments.

At the whole plant level field experiments were conducted concurrently with the development of a biochemical photosynthesis model. The experimental work was designed to investigate short term response to a sudden change in CO_2 concentration and long term adaptation to distinctive environmental CO_2 levels. All of the treatments were exposed to constant moderate air temperatures under well-fertilized and well-watered conditions.

The experiments were conducted under natural sunlight which varied, but all treatments were exposed equally to these changes.

To assess the plant-environment interactions four general classes of data are required: (1) external parameters such as temperature, quantum flux density and CO_2 concentration which define the environment; (2) gas exchange rates such as transpiration, daytime CO_2 exchange and night time CO_2 exchange which define water use, photosynthesis and respiration rates; (3) whole plant parameters such as height, leaf area and biomass which are indicators of adaptive response; and finally, (4) physiological parameters such as specific leaf weight, chlorophyll levels and nitrogen levels which are indicators of biochemical system response. The short and long-term adaptive response of plants to a particular sequence of prevailing environments can be characterized in terms of these four data sets.

At the biochemical level the experimental results can be applied to questions concerning substrate levels and process rates on the micro-scale. For instance, the data can suggest how CO_2 level effects chlorophyll concentrations and enzyme levels (as a function of nitrogen) as well as CO_2 fixation process rates. On a larger scale the data can indicate how source-sink balancing is affected by CO_2 concentration through the measurements of diurnal specific leaf weight and by the instantaneous measurements of photosynthetic light response. Finally, at the whole plant level, biomass accumulation and leaf area are direct measures of the integrated adaptive response of whole plants to different CO_2 environments.

The experimental work was designed to provide the data necessary to answer these questions. With these data, the hypothesis that plants

adaptively respond to different prevailing environmental concentrations of CO_2 can be tested. Furthermore, the adjustments that occur at the whole plant level and the biochemical level can be considered separately, to determine whether the adaptation of the whole plant is consistent with biochemical level response.

Combining the experimental results with the biochemical photosynthesis model, a qualitative explanation of short-term feedback controlled response and long-term whole plant adaptation to prevailing CO_2 concentrations is proposed. This proposed mathematical-conceptual model provides a framework for both a short-term model to explain inhibition and enhancement of photosynthetic light response and a long-term model of adaptation to differing environments.

The overall purpose of this research is based on the concept of photosynthetic adaptation which has been defined as environmentally induced adjustments in physiology, anatomy and morphology that allow a plant to improve photosynthetic efficiency in a new environment [Bjorkman and Berry, 1973]. Stated another way, adaptation enables whole plants to maximize photosynthetic productivity under locally prevailing environmental conditions [Tooming, 1970]. The purpose of this research is to gain a more complete understanding of photosynthesis as a dynamic process at both the whole plant and biochemical levels.

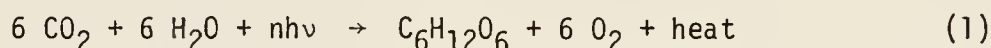
It is postulated that plant canopies will adaptively respond to different prevailing environmental concentrations of carbon dioxide. In particular, this project has been designed to describe and explain the responses of soybean canopies grown continuously in different carbon dioxide concentrations during their vegetative stage of growth and the

subsequent short and long-term canopy responses to a step change in CO₂ levels. The experimental objective of the research has been to grow and monitor soybean canopies in four computer controlled, closed environmental chambers. A parallel theoretical objective has been to develop a biochemical level model of photosynthesis. Finally, the third objective of the research has been to qualitatively relate the whole plant experimental observations and the biochemical model within the conceptual framework of source-sink balancing.

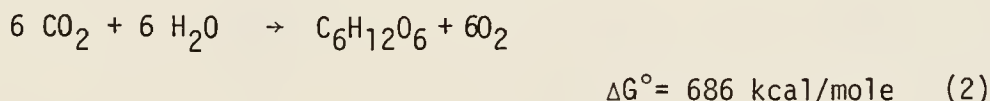
The long range goal of this research is to devise a physically based dynamic model of photosynthesis complete with feedback controls. Such a model could be used with other sub-system models to describe any whole plant system. In turn individual plant models are the basis of crop models which have increasingly wide application.

BIOCHEMICAL PHOTOSYNTHESIS MODEL

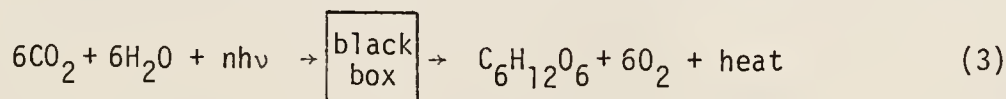
Photosynthesis is commonly modeled [Lehninger, 1970] as a process in which carbon dioxide (CO_2) and water (H_2O) are chemically combined in the presence of light quanta ($nh\nu$) to form glucose ($\text{C}_6\text{H}_{12}\text{O}_6$) and oxygen (O_2). In equation form this is expressed as:



Note that mass is conserved explicitly in the equation stoichiometry, whereas energy is implicitly conserved by equating light input ($nh\nu$) to the chemical energy in glucose ($\text{C}_6\text{H}_{12}\text{O}_6$) and the energy degraded to heat. It is useful to rewrite equation (1), replacing the light quanta term ($nh\nu$) with an associated free energy change (ΔG).



This emphasizes that in the formation of glucose, light is not a substrate, but rather, indirectly supplies the energy to drive this "uphill" reaction. Finally, a third equation can be written to make the obvious point that carbonated water exposed to sunlight will not produce glucose and oxygen. CO_2 and water must pass through an extensive series of biochemical cycles and pathways before being transformed into glucose; therefore, equation (1) might be written once again:

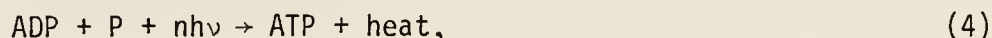


In the following sections, some of the details of the photosynthetic blackbox will be discussed, simplified and condensed into a mathematical model.

The Light Reactions

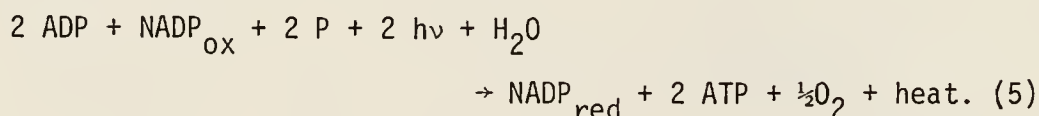
When photosynthesis is considered in detail, equation (1) is seen to incorporate two chemical processes which are coupled into the total system by which glucose is formed. Light is required in an initial process in which photosynthetically active radiation (PAR) is converted into chemical energy via excitation of chlorophyll and accessory pigment molecules. These initial reactions are called the Light Reactions. PAR is electromagnetic radiation with wavelength between 400 and 700 nm. It is usually measured with a quantum sensor and typical units are micro-Einsteins/m²/sec. PAR quanta are absorbed by a diverse group of pigments located in the chloroplasts of photosynthesizing cells. The most commonly known and abundant pigment is chlorophyll, of which there are several kinds that differ slightly in structure and absorption spectrum. Chlorophyll is the main light absorbing pigment in green plants. When plants are exposed to PAR, quanta are absorbed, causing high energy electrons to escape from the excited pigment molecules. Some of these electrons fall back to ground state and the chlorophyll molecules give up their captured quanta as fluorescence and heat. Others leave the chlorophyll completely and enter an electron-carrier pathway, flowing down an energy gradient from one carrier to the next. When an electron moves through this transport system, it loses potential energy at each transfer between carriers. At certain of the transfers in the chain, the potential drop is partially conserved by driving the energy requiring phosphorylation of adenosine diphosphate (ADP) to adenosine triphosphate

(ATP). Two quanta are absorbed for each electron to move through the complete pathway. In this manner light-induced electron flow is converted to chemical bond energy. The process is called photophosphorylation and can be represented by the following equation:

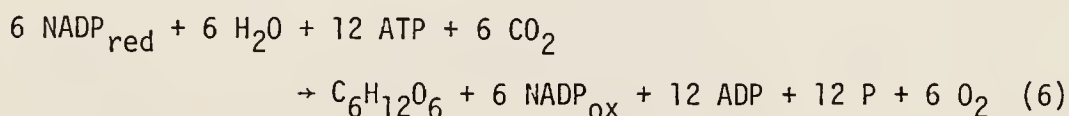


where P is inorganic phosphate.

Only part of the photoinduced potential is used to produce ATP. Most of the conserved electrochemical energy goes to the last acceptor in the chain, which is the oxidized form of nicotinamide adenine dinucleotide phosphate (NADP) which receives the electron as well as a proton and is accordingly reduced as the final step in the light reactions. This process of using photoinduced electron flow to yield a reduced product is called photoreduction. Equation (4) can be expanded to represent the overall light reaction, including both photophosphorylation and photoreduction as follows [Lehninger, 1973]:



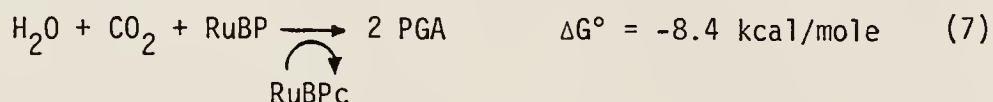
With this more detailed understanding, equation (1) can be rewritten again to clarify the relationship between PAR as an energy source and the glucose formation equation. It is



Equation (6) describes an overall process which has been only briefly outlined. More complete descriptions of the light reactions are available in articles by Rabinowitch and Godwinjee [1965] and Zelitch [1979].

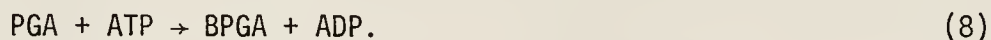
Dark Reactions: Calvin Cycle

The biochemical pathway to sucrose following the light reactions is referred to as the Dark Reaction. A central portion of this process is the Calvin cycle, which occurs in the chloroplast, along with the light reactions. The first step in the Calvin cycle is the reduction of CO_2 (the carboxylation reaction). Specifically, the reaction combines CO_2 , H_2O and ribulose-1,5-bisphosphate (RuBP) in the presence of the enzyme ribulose-1,5-bisphosphate carboxylase (RuBPc) to form 2 molecules of 3-phosphoglyceric acid (PGA). In equation form it is

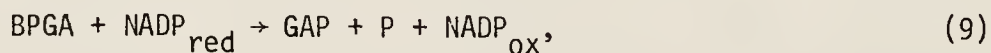


Equation (7) is an exergonic or downhill reaction having a negative standard free energy change [Bassham, 1971] and, therefore, requires no energy or reducing power to proceed. Although not directly required in equation (7), the light reaction's products (ATP and NADP_{red}) drive the complex sequence of enzyme catalyzed reactions called the Calvin cycle, which regenerates RuBP [Bassham, 1971].

In the second reaction of the Calvin cycle, ATP is directly required for the phosphorylation of PGA, producing the high energy phosphate compound 1,3-biphosphoglyceric acid (BPGA):



The reducing power generated by the light reaction is utilized in the subsequent step as follows:



where GAP is glyceraldehyde-3-phosphate. The only other reaction in

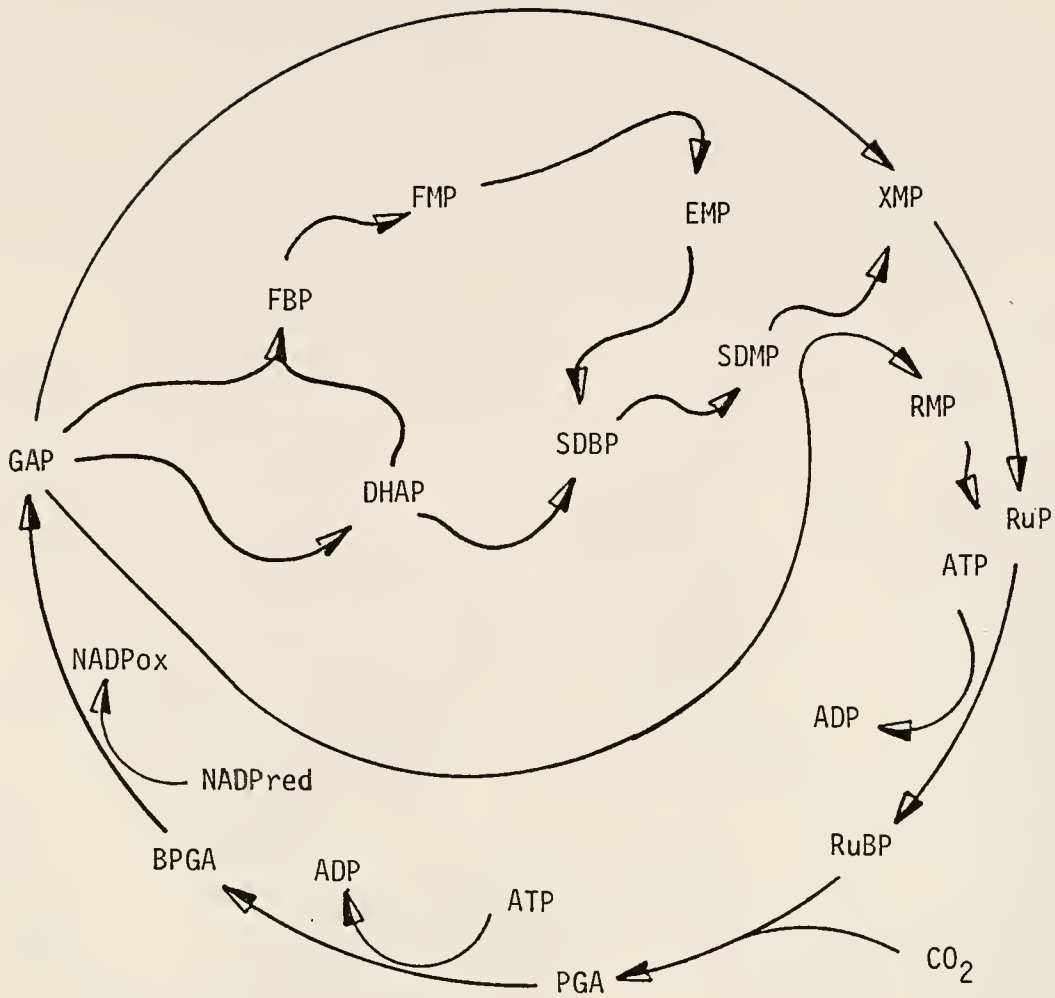
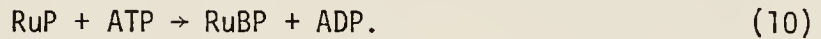


Figure 2. Calvin cycle schematic. RuBP, ribulose-1,5 -bisphosphate, RuP; ribulose-5-phosphate; XMP xylulose-5-phosphate; SDMP, sedoheptulose-1-phosphate; SDBP, sedoheptulose-1,7-bisphosphate; EMP erythrose-4-phosphate; FMP, fructose-6-phosphate; FBP, fructose-1,6-phosphate; DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde-3-phosphate; BPGA, 1,3-bisphosphoglyceric acid; PGA, 3-phosphoglyceric acid. All abbreviations are listed alphabetically in Appendix 1 (Based on Bassham [1971]).

the Calvin cycle directly using the products of the light reaction is step 13 in Table (1), in which ribulose-5-phosphate (RuP) is phosphorylated to RuBP:



The preceding three equations summarize the direct interaction between the Calvin cycle and the light reactions, while equation (7) represents the all important link between the microscale biochemical pathways and the macroscale carbon exchange rates.

Dark Reactions: Phosphate Translocator System

Both the light reactions and the Calvin cycle occur in chloroplasts while the final steps in the dark reactions take place in the cytoplasm, requiring that the carbon fixed in equation (7) be exported through the outer chloroplastic membrane. The primary export product is dihydroxyacetone phosphate (DHAP) [Heber, 1974], which is produced in reversible equilibrium with GAP as the fourth step in the Calvin cycle. For this pathway to function continuously, cytoplasmic phosphate must be imported to the chloroplast in direct proportion to the exported DHAP. The exchange is part of the phosphate translocator system. DHAP is a crucial intermediate which is balanced among three pathways: export for use as substrate or energy in the cytoplasmic dark reactions, continuation in the Calvin cycle as substrate for regenerating RuBP, or to storage as starch in the chloroplast. A schematic is given in Figure (3) to clarify these relationships. The other portion of the system imports PGA from the cytoplasm in exchange for chloroplastic inorganic phosphate [Kelly et al., 1976]. The importance of maintaining phosphate balances is clear, considering its role in energy transfer and storage. Once

Table 1. Calvin cycle reactions (H_2O not shown).

Step	Substrate	Enzyme	Product
1	6 CO_2 + 6 RuBP	RuBP carboxylase	12 PGA
2	12 PGA + 12 ATP	PGA kinase	12 BPGA + 12 ADP
3	12 BPGA + 12 NADP_{red}	GAP dehydrogenase	12 GAP + NADP_{ox}
4	5 GAP	Triose phosphate isomerase	5 DHAP
5	3 GAP + 3 DHAP	Aldolase	3 FBP
6	3 FBP	FBP dekinase	3 FMP + 3 P
7	2 FMP + 2 GAP	Transketolase	2 XMP + 2 EMP
8	2 EMP + 2 DHAP	Aldolase	2 SBP
9	2 SBP	Phosphatase	2 SMP + 2 P
10	2 SMP + 2 GAP	Transketolase	2 RMP + 2 XMP
11	2 RMP	Isomerase	2 RuP
12	4 XMP	Epimerase	4 RuP
13	6 RuP + 6 ATP	Phosphoribulokinase	6 RuBP + 6 ADP

Note: Reactions are taken from Lenhinger [1970].

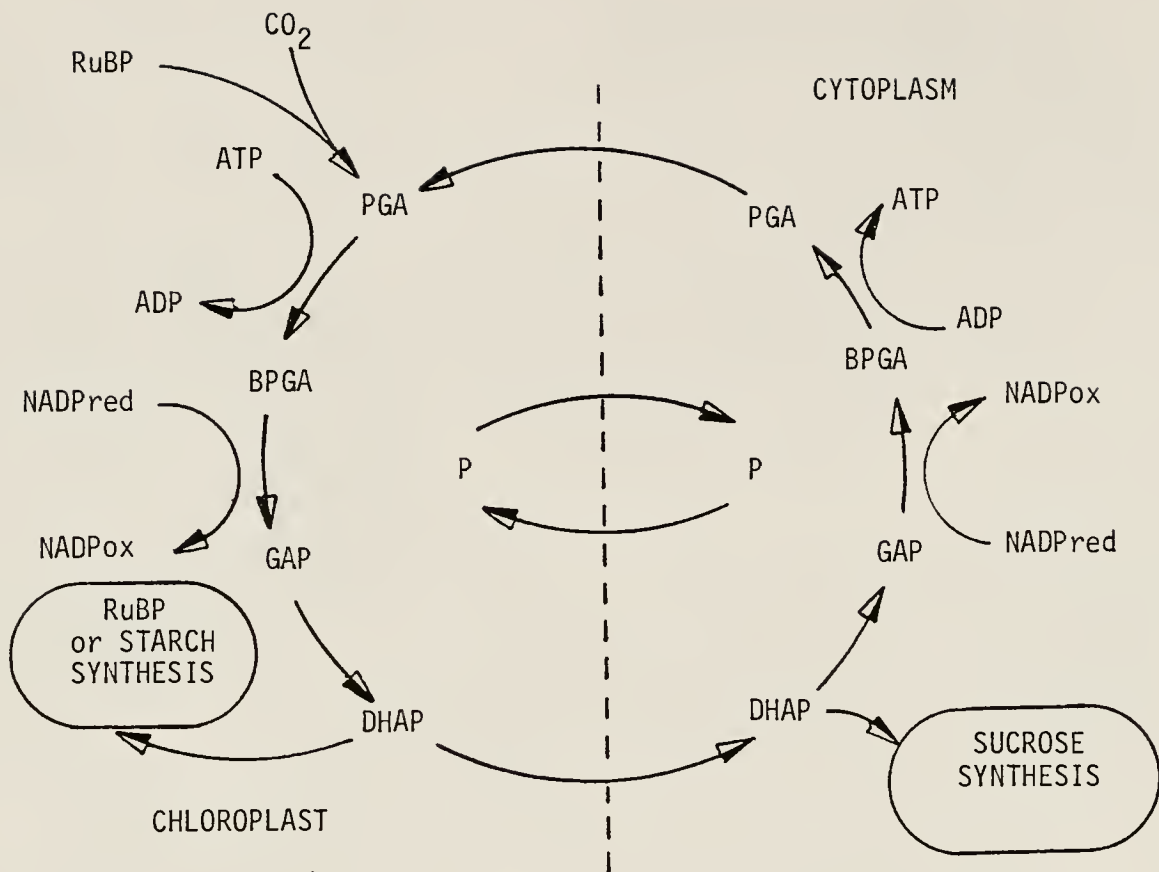


Figure 3. Phosphate translocator system schematic. Outlines the membrane exchange mechanism by which fixed carbon is exported from chloroplasts to cytoplasm for sucrose synthesis. All abbreviations are identified in Appendix 1 (Based on Heber [1974]).

DHAP is in the cytoplasm, it can be utilized as a substrate in the sucrose pathway or it can be oxidized and dephosphorylated by the reverse reactions of equations (9) and (10). In this way the energy equivalents of ATP and NADP_{red} are transported to the cytoplasm. When used for energy transfer, the DHAP is converted to PGA, which can be transported back to the chloroplast [Herold and Walker, 1979].

Dark Reactions: Chloroplastic Starch Pathway

In the chloroplast, the starch pathway is cyclical, moving from DHAP to starch, and later being reconverted to DHAP. The intermediate steps have been worked out in detail and are presented in Table (2). The cycle actually moves through steps (5) and (6) of the Calvin cycle in which fructose-1,6-bisphosphate (FBP) is irreversibly converted to fructose-6-phosphate (FMP), which enters the starch pathway, forming glucose-6-phosphate (G6P). The rate limiting step in starch formation is the reaction in which ATP combines with glucose-1-phosphate (G1P) to form ADP-glucose. This reaction is not only promoted by high levels of ATP, but also by high levels of PGA, and is inhibited by high levels of inorganic phosphate [Kaiser and Bassham, 1979]. The return starch mobilization reactions follow the same essential pathway, except that the rate limiting step is the energy requiring conversion of FMP to FBP. This reaction obtains energy and a phosphate group from ATP, while simultaneously being inhibited by high levels of ATP.

The system behavior described acts as a regulator encouraging starch storage during the day and release at night. Also note that energy units are required in both the formation and breakdown of starch. A schematic of this cycle is given in Figure (4).

Table 2. Chloroplastic starch cycle reactions.

Step	Substrate	Enzyme	Product
1	DHAP + GAP	Aldolase	FBP
2	FBP	Dekinase	FMP + P
3	FMP	Phosphohexoisomerase	G6P
4	G6P	Phosphoglucomutase	G1P
5	G1P + ATP	Pyrophosphorylase	ADP-glucose + P
6	ADP-glucose	Amylose synthetase	Starch
7	Starch + P	Glucan phosphorylase	G1P
8	G1P	Phosphoglucomutase	G6P
9	G6P	Phosphohexoisomerase	FMP + ADP
10	FMP + ATP	Phosphofructokinase	FBP
11	FBP	Aldolase	DHAP + GAP

Note: These reactions are based on Lehninger [1970] and Kelly et al. [1976].

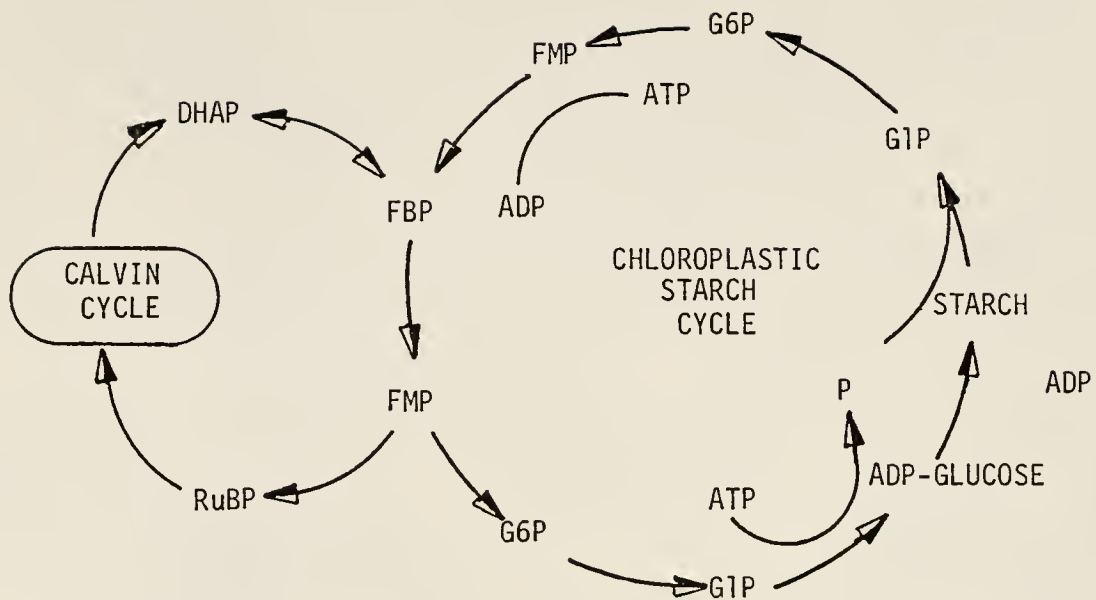


Figure 4. Chloroplastic starch cycle schematic. Outlines the starch storage and remobilization mechanism by which chloroplast can store fixed carbon. All abbreviations are listed in Appendix 1 (Based on Kelly et al. [1976]).

Dark Reactions: Cytoplasmic Sucrose Pathway

The other carbon balancing pathway is to the cytoplasm, where DHAP is the initial substrate leading to sucrose formation. In a series of steps similar to those in starch formation, DHAP is converted to glucose-1-phosphate (G1P). At this point, sucrose formation diverges from the starch pathway. Instead of the common energy compound ATP, G1P combines with uridine triphosphate (UTP) to form UDP-glucose. This compound reacts with FMP to form sucrose-6-phosphate (SMP), which directly yields sucrose. One important aspect of this pathway is that the reaction rate of the SMP conversion to sucrose may be subject to product inhibition [Hawker, 1967]. Sucrose is the primary sugar compound exported from photosynthesizing cells. If the export of sucrose is less than its synthesis, then cytoplasmic levels will increase. When this happens, the high concentration of sucrose potentially inhibits the enzyme that dephosphorylates SMP, causing a buildup of its concentration and a corresponding decrease in the level of inorganic phosphate. This pathway is detailed in Table (3), and a schematic is presented in Figure (5). Another point to consider is that uridine triphosphate (UTP) formation is driven by ATP, and that the roles of these compounds in carbohydrate formation are very similar.

In review, equation (1) is seen to represent substrate and energy fluxes into the photosynthetic blackbox on the left hand side and product fluxes out of the system on the right hand side. To better understand this equation, the blackbox has been described in terms of five sub-systems:

1. the light reaction
2. the Calvin cycle
3. the phosphate translocator system
4. the chloroplastic starch cycle
5. the cytoplasmic sucrose pathway

Table 3. Cytoplasmic sucrose pathway reactions.

Step	Substrate	Enzyme	Product
1	DHAP + GAP	Aldolase	FBP
2	FBP	Dekinase	FMP + P
3	FMP	Phosphohexoisomerase	G6P
4	G6P	Phosphoglucomutase	G1P
5	G1P + UTP	Pyrophosphorylase	UDPG + PP
6	UDPG + FMP	Phosphosynthetase	SMP + UDP
7	SMP	Phosphatase	Sucrose + P

Note: PP is an abbreviation for pyrophosphate. Reactions are based on Lehninger [1970] and Kelly et al. [1976].

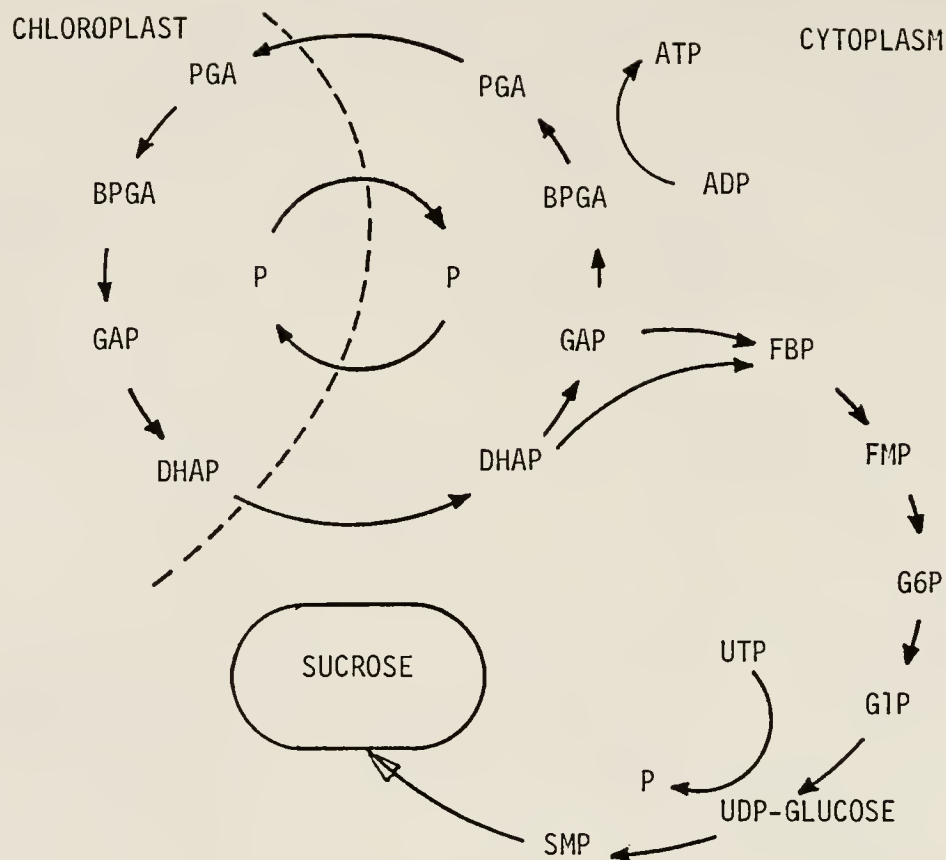


Figure 5. Cytoplasmic sucrose pathway schematic. Abbreviations are defined in Appendix 1 (Based on Lehninger [1970] and Kelly et al. [1976]).

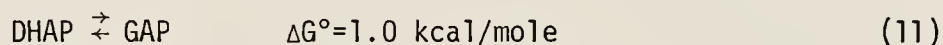
From the schematics and equations given to describe these processes, the fluxes in equation (1) are plainly visible. The light reactions use captured quanta ($nh\nu$) to drive the hydrolysis of water (H_2O), which supplies electrons for use in the reduction of NADP and simultaneous formation of ATP. Oxygen (O_2) is liberated as a by-product of hydrolysis. These interactions are described in equation (5). Carbon dioxide (CO_2) enters the system reacting with RuBP to form the first products in the Calvin cycle, according to equation (7). Fixed carbon is transported from chloroplast to cytoplasm where sucrose is formed and exported from the cell. Some fixed carbon is temporarily stored in the chloroplast as starch and later is transported to the cytoplasm, where it also forms sucrose. The model development emphasizes the feedback regulation of the carbon flow paths into and out of the photosynthetic cell.

Simplifying Assumptions

As can be seen from the preceding tables and schematics, there are a large number of intermediates involved in each subsystem. In the model being developed, the concentration of intermediates fluctuates in response to net flow. It is assumed that keeping track of each intermediate pool is not necessary because of the serial nature of the processes. To aid in determining which intermediates should be retained, mediating enzymes have been grouped according to their characteristics as given by Kelly et al. [1976], Heber [1974] and Lehninger [1970]. Essentially, all biochemical reactions on the cellular level are catalyzed enzymatically, each enzyme may or may not be inhibited or promoted by any other compound or ion. Enzyme, product and substrate groups are also unique in their degree of biochemical reversibility, which will vary with pH and other factors. In short, reactions can range from very simple reversible flow between two proportionate

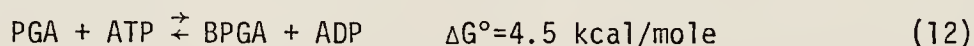
pools to irreversible reactions which are highly sensitive to a range of inhibitors and promoters. For purposes of modelling, it is essential to sort out the reactions which may be crucial rate limiting and path selecting points from those that are not. Four criteria have been devised to sort out the significant interactions in each subsystem.

The first simplifying criterion is that nonallosteric enzymes can be ignored and that reversible path reactions between a substrate pool and a product pool can be condensed into a single representative tank. An excellent example of this situation is the fourth step in the Calvin cycle, in which DHAP and GAP quickly reach an equilibrium because of the continuous availability of active enzyme [Lehninger, 1970].



In modelling this portion of the Calvin cycle, no distinction needs to be made between these triose phosphates; the presence of one implies the presence of the other in some approximately constant ratio. Since DHAP has a significant role in path selection, the representative storage pool is referred to as DHAP.

The second criterion extends the conditions of the first to include nonallosteric reactions which require energy or reducing units. The second Calvin cycle reaction fits these requirements.



In the chloroplast exposed to light, ATP levels are high and equation (12) can be expected to have a net flow to the right, while in the dark, the flow will reverse direction (see postscript on PGA).

The third Calvin cycle reaction is similar to the second, requiring reduced NADP to proceed from BPGA to GAP. The reactions described so far

under the first and second criteria are modelled schematically in Figure (6), using "Energy Language Symbols" developed by H.T. Odum [1971]. There are four predominate symbols used: the tank which symbolizes the concentration of a metabolite in the reaction medium; the interaction symbol which relates substrates and/or allosteric effectors; the circle which represents an unlimited flow source, and the interaction arrow, which shows the explicit path taken (see Appendix IV).

Looking at Figure (6), BPGA and GAP can be consolidated into a flow path between PGA and DHAP which is moderated by the levels of ATP and NADP_{red} . The process can be further simplified by assuming that reducing power and energy units come from the same source in a reasonably constant ratio. Hence, if ATP is available, then NADP_{red} should also be available. The condensed model is shown schematically in Figure (7).

A third criterion treats the class of nonallosteric reactions which have products. An example is the dephosphorylation of FBP to form FMP and inorganic phosphate, P, which occurs in the Calvin cycle as well as the cytoplasmic sucrose pathway. This process can be modelled schematically as in Figure (8). The assumption is that tanks can be condensed, but the product P cannot be ignored. FBP can be absorbed into the DHAP tank and FMP can be absorbed into the G6P tank according to the first criterion, while P must flow to an inorganic phosphate tank. The simplified schematic version is in Figure (9).

The fourth criterion deals with allosteric rate limited reactions by controlling flow between pools with elements that sense reaction inhibitors and/or promoters. In the starch forming reaction, step 6 in Table (2), the enzyme is inhibited by inorganic phosphate (P) and promoted by the intermediate, PGA [Kaiser and Bassham, 1979]. So, even

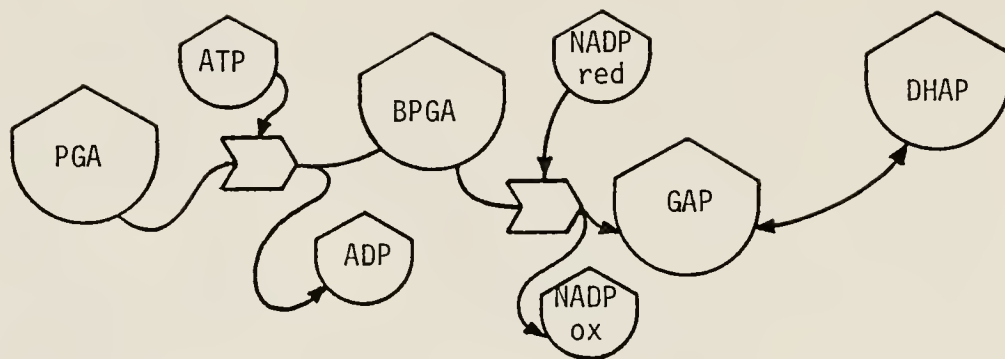


Figure 6. PGA-DHAP pathway schematic. Explicit equations are listed in Table (1).

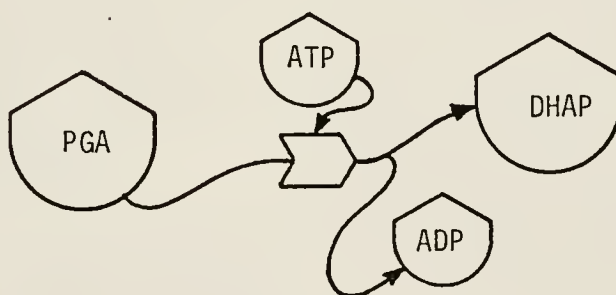


Figure 7. Simplified PGA-DHAP pathway. All abbreviations are listed in Appendix 1. (Symbols are defined in Appendix 4.)

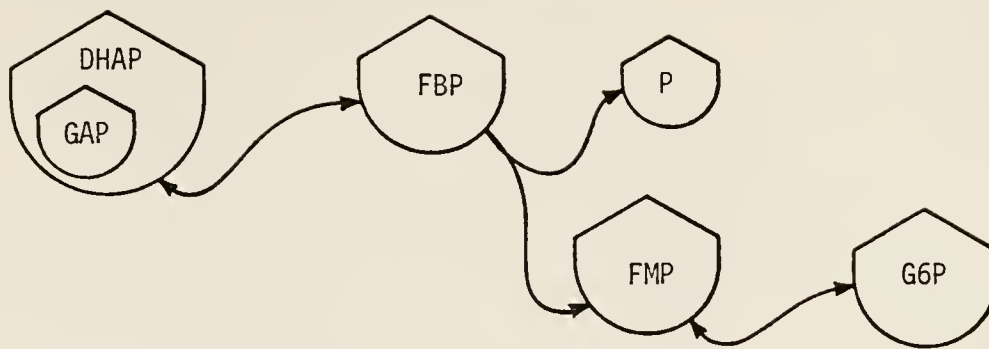


Figure 8. DHAP-G6P pathway schematic. Explicit equations are in Table (2).

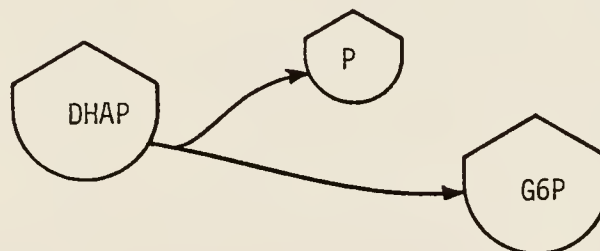


Figure 9. Simplified DHAP-G6P pathway schematic. All abbreviations are defined in Appendix 1. (Symbols are defined in Appendix 4.)

though these compounds are not substrates in the reaction, they must be included in some form to control the reaction.

The application of these criteria to the five subsystems results in the simplified model shown schematically in Figure (10). As shown below, the schematic can be used to generate differential equations by doing simple mass flow balances into and out of each tank.

Differential Equation Development

The interaction symbol between tanks indicates a simple algebraic process involving the concentrations of reactants and a rate constant. The level of PGA1 in the chloroplast is expected to vary according to

$$\frac{d[\text{PGA1}]}{dt} = 2k_1 [\text{RuBP}] [\text{CO}_2] + k_2 [\text{PGA2}] [\text{P1}] - k_3 [\text{PGA1}] [\text{ATP1}]. \quad (13)$$

The first term on the right hand side of equation (13) represents an addition to the PGA1 pool resulting from the reduction of CO_2 which is represented as a function of the concentrations of CO_2 and RuBP as moderated by the rate constant k_1 . The 2 is a numerical constant stoichiometrically required to maintain the system's carbon balance. The second term is the phosphate translocator flow of PGA from the cytoplasm to chloroplast as a function of [PGA2] and [P1], multiplied by the rate constant, k_2 . The third term is the Calvin cycle forward flow to DHAP, a function of [PGA1] and available energy units, [ATP1]. Notice that the third term embodies the assumptions outlined in Figures (6) and (7).

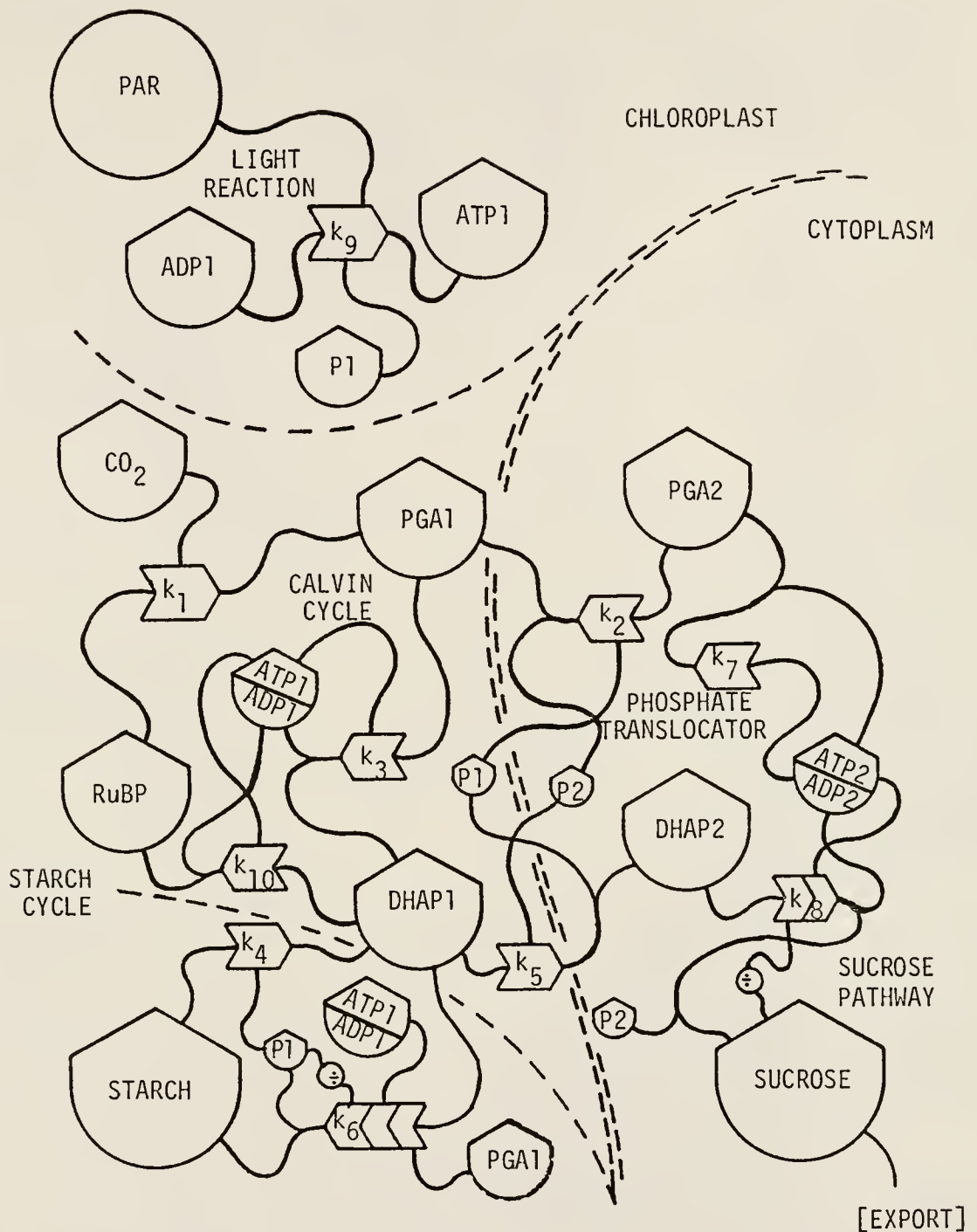


Figure 10. Simplified overall photosynthesis schematic. In the interaction symbols the k values are reaction rate constants. The divisor sign implies inhibition. Numbers following intermediates separate chloroplastic (1) from cytoplasmic (2) pools. All abbreviations are defined in Appendix 1. (Symbols are defined in Appendix 4.)

Other equations derived from the schematic are

$$\begin{aligned} \frac{d[\text{DHAP1}]}{dt} = & k_3 [\text{PGA1}] [\text{ATP1}] + 2k_4 [\text{STARCH}] [\text{P1}] \\ & - k_{10} [\text{DHAP1}] [\text{ATP1}] - k_5 [\text{DHAP1}] [\text{P2}] \\ & - k_6 [\text{DHAP1}] [\text{PGA1}] [\text{ATP1}]/[\text{P1}] \end{aligned} \quad (14)$$

$$\begin{aligned} \frac{d[\text{STARCH}]}{dt} = & \frac{1}{2}k_6 [\text{DHAP1}] [\text{PGA1}] [\text{ATP1}]/[\text{P1}] \\ & - k_4 [\text{STARCH}] [\text{P1}] \end{aligned} \quad (15)$$

$$\begin{aligned} \frac{d[\text{DHAP2}]}{dt} = & k_5 [\text{DHAP1}] [\text{P2}] \\ & - k_7 [\text{DHAP2}] [\text{ADP2}] - k_8 [\text{DHAP2}] [\text{ATP2}]/[\text{SUCROSE}] \end{aligned} \quad (16)$$

$$\frac{d[\text{PGA2}]}{dt} = k_7 [\text{DHAP2}] [\text{ADP2}] - k_2 [\text{PGA2}] [\text{P1}] \quad (17)$$

$$\frac{d[\text{SUCROSE}]}{dt} = \frac{1}{4}k_8 [\text{DHAP2}] [\text{ATP2}]/[\text{SUCROSE}] - [\text{EXPORT}] \quad (18)$$

$$\frac{d[\text{RuBP}]}{dt} = \frac{3}{5}k_{10} [\text{DHAP1}] [\text{ATP1}] - k_1 [\text{RuBP}] [\text{CO}_2]. \quad (19)$$

In addition to DHAP and PGA, separate tanks in chloroplast and cytoplasm are maintained for ATP, ADP, and P. Their differential equations are

$$\frac{d[ATP1]}{dt} = k_9 [LIGHT] [ADP] [P1] \quad (20)$$

$$- k_3 [PGA1] [ATP1] - k_4 [STARCH] [P1]$$

$$- k_6 [DHAP1] [PGA1] [ATP1]/[P1]$$

$$- k_{10} [DHAP1] [ATP1]$$

$$\frac{d[ADP1]}{dt} = - \frac{d[ATP1]}{dt} \quad (21)$$

$$\frac{d[P1]}{dt} = k_5 [DHAP1] [P2] + 2k_6 [DHAP1] [PGA1] [ATP1]/[P1] \quad (22)$$

$$- k_4 [STARCH] [P1] - k_2 [PGA2] [P1]$$

$$- k_9 [LIGHT] [ADP] [P1]$$

$$\frac{d[ATP2]}{dt} = k_7 [DHAP2] [ADP2] \quad (23)$$

$$- k_8 [DHAP2] [ATP2]/[SUCROSE]$$

$$\frac{d[ADP2]}{dt} = - \frac{d[ATP2]}{dt} \quad (24)$$

$$\frac{d[P2]}{dt} = k_2 [PGA2] [P1] + 2k_8 [DHAP2] [ATP2]/[SUCROSE] \quad (25)$$

$$- k_5 [DHAP1] [P2].$$

Some terms require numerical constants in order to maintain the system's carbon and phosphate balances.

Most of the terms in these equations are concentration driven in the sense that higher substrate or cofactor levels increase the probability of a reaction. Allosteric effects should be distinguished; in equation (14), the last term, PGA_1 , behaves like the substrate $DHAP_1$, but its effect is to promote the enzyme which catalyzes the reaction. In the same term, P_1 acts to allosterically inhibit the process rate. The algebraic form of the inhibitory effect is arbitrary in this equation. Different responses could be obtained with different algebraic arrangements. Without in vivo response curves, a very simple feedback response has been chosen.

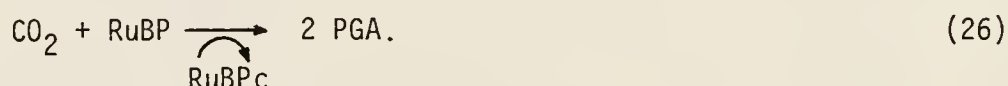
In the schematic given in Figure (10), the components of equation (1), which flow into and out of the blackbox, are each treated differently. This reflects certain assumptions about the paths which each input and output to the photosynthesizing system must follow. The source of the PAR is represented schematically as a circle rather than a tank, since the absorption of quanta has no effect on the rate of quanta arriving at the active site. In contrast, CO_2 is a tank placed inside the chloroplast, implying that concentration of available CO_2 is affected by the rates of CO_2 arrival and exit at the active site. Water is ignored, since it is always present in abundance for chemical reaction, even when the plant is under water stress. Oxygen which is liberated in the chloroplast is also ignored because it has only a minor impact on the high levels of O_2 already present due to diffusion from the atmosphere. Finally, sucrose (carbohydrate) is exported according to some arbitrary function with a dependence on sucrose concentration.

The most glaring shortcoming of the model developed so far involves the first step in the Calvin cycle. This is the rate limiting reaction for the entire system. As such, it must be considered in more detail.

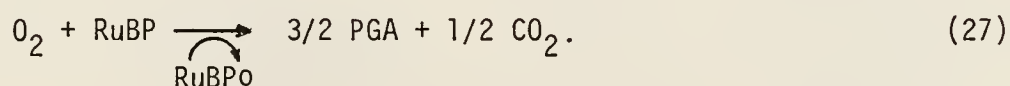
A second major omission from the model is the competitive oxygenase reaction, photorespiration, which is closely related to the first Calvin cycle step, the carboxylase reaction.

Carboxylation, Photorespiration and Enzyme Kinetics

As described in equation (1), the first reaction in the Calvin cycle is the fixation of CO_2 , which can be abbreviated for purposes of a Michaelis-Menten style analysis to the following



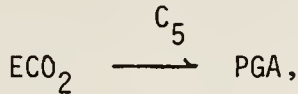
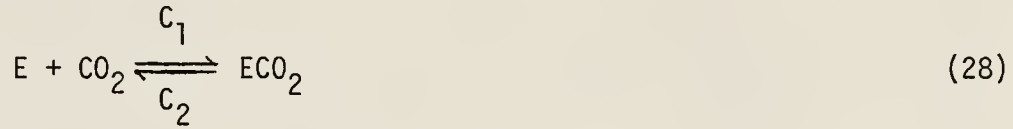
Water has been eliminated since it is always at saturation levels and does not affect the reactions rate. In competition with this reaction is photorespiration, which can be written as



Equation (27) is not strictly chemically correct as written. Actually, phosphoglycolate is a product of this reaction that goes on to produce CO_2 and PGA, which are products of interest in this model. Both of these reactions utilize the same enzyme, called ribulose biphosphate carboxylase (RuBPC) in equation (26) and ribulose biphosphate oxygenase (RuBPo) in equation (27). In addition, both reactions require the same second substance, RuBP, which is often assumed to be at saturating concentrations [Jensen et al., 1978], allowing it to be ignored, like water. With this assumption, both reactions above can be approximated by first order processes which can be analyzed with enzyme kinetic theory.

Consider the reactions to be occurring in a well mixed medium, with concentrations of carbon dioxide substrate, $[\text{CO}_2]$, oxygen substrate,

$[O_2]$, ribulose biphosphate substrate [RuBP] and activated ribulose biphosphate carboxylase-oxygenase enzyme (E). Assume that the enzyme reacts with either CO_2 or O_2 as a function of the activated enzyme's relative affinity for the two substrates and the temperature adjusted relative concentrations of the two substrates. Under the assumption that RuBP can be ignored because it is at saturation levels, the carboxylation reaction equations are



where C_1 , C_2 and C_5 are reaction rate constants, and ECO_2 is the enzyme substrate complex. These equations can be manipulated to yield a Michaelis-Menten equation of the following form:

$$V_c = \frac{V_{cmax} [CO_2]}{[CO_2] + K_c}, \quad (29)$$

where V_c is the rate of substrate CO_2 utilization, V_{cmax} is the maximum rate of substrate CO_2 utilization and K_c is the concentration of substrate CO_2 that will sustain the reaction at 1/2 the maximum rate. The corresponding equation for the oxygenase reaction is

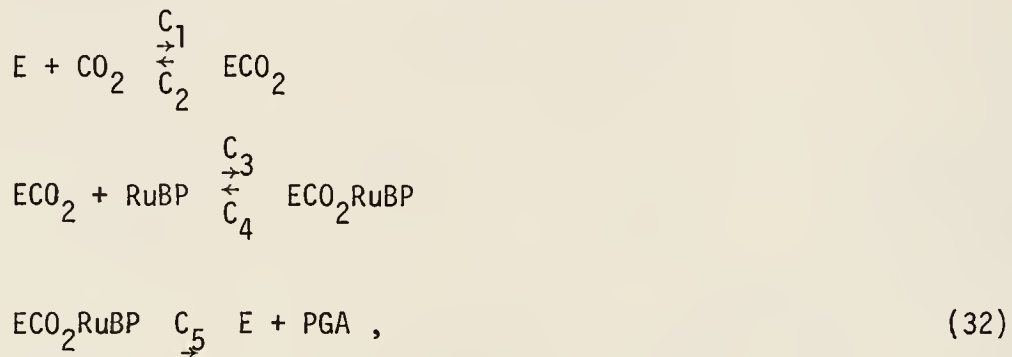
$$V_o = \frac{V_{omax} [O_2]}{[O_2] + K_o}, \quad (30)$$

where the variables are analogous to those in the preceding equation. Given that both of these "first order" reactions use the same enzyme and assuming that neither substrate affects the affinity of the enzyme for

the other, they can be treated as a classic case of competitive inhibition. The resulting equation for carboxylation which includes this competitive effect is

$$V_{co} = \frac{V_{comax} [CO_2]}{[CO_2] + K_c (1 + [O_2]/K_o)} \quad (31)$$

If RuBP is not assumed to be at saturating levels and is included in the analysis, the carboxylation equations must be expanded to include an intermediate reaction as follows:



where C_2 and C_4 are reaction rate constants and ECO_2RuBP is a second stage substrate-enzyme complex. Assuming that each substrate combines with its specific site without either substrate affecting the enzyme's affinity for the other, then the carboxylation equation is

$$V_{cr} = V_{crmax} \left(\frac{[CO_2]}{[CO_2] + K_c} \right) \left(\frac{[RuBP]}{[RuBP] + K_r} \right). \quad (33)$$

As before, parallel development yields the following oxygenase equation:

$$V_{or} = V_{ormax} \left(\frac{[O_2]}{[O_2] + K_o} \right) \left(\frac{[RuBP]}{[RuBP] + K_r} \right). \quad (34)$$

These relationships describe the flow rates among the reactant and product pools as shown in Figure (11).

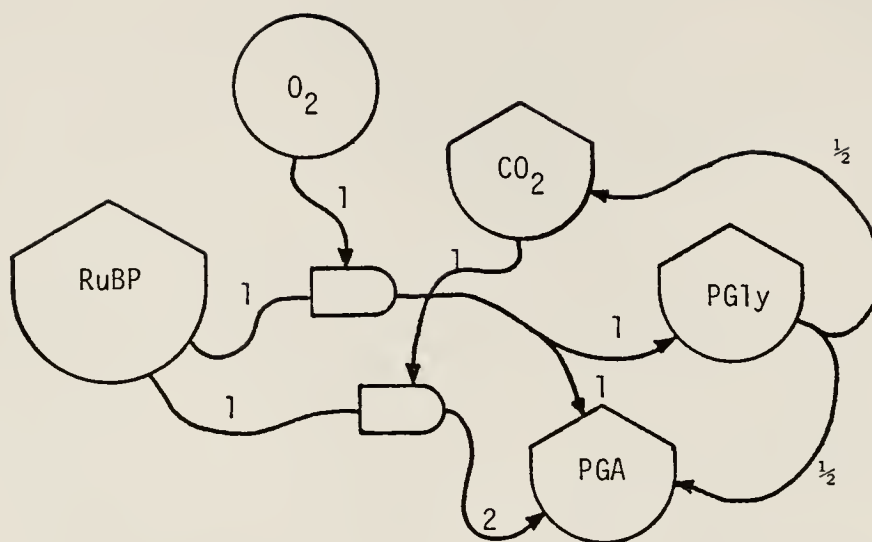


Figure 11. Carboxylase-oxygenase pathway schematic. This schematic models the competition by O_2 and CO_2 for RuBP. The numbers represent stoichiometric balances for each path and the new interaction symbol represents a Michaelis-Menten function.

From the equations developed, it is postulated that the rate of photosynthesis is a function of the substrate concentrations within the stroma. The carboxylase reaction is regulated by the concentration of CO_2 at the reaction site, while the oxygenase reaction is a function of O_2 concentration and both processes depend on the RuBP level. Since both pathways draw on the available supply of RuBP, the steady state level is lowered, which effectively depresses both reaction rates [Canvin, 1978].

The effect of this analysis on the model is to change the functional form of the flows among the tanks involved in the first Calvin cycle reaction. This involves the differential equations for PGA1 and RuBP, which are numbers (13) and (19), respectively. Instead of the simple algebraic product of concentrations, equations (33) and (34) are used to model flows from the RuBP tank and to the PGA tank. The new balance equations are

$$\begin{aligned} \frac{d[\text{PGA1}]}{dt} = & 2 V_{\text{cmax}} \left(\frac{[\text{CO}_2]}{[\text{CO}_2] + K_{\text{c}}} \right) \left(\frac{[\text{RuBP}]}{[\text{RuBP}] + K_{\text{r}}} \right) \\ & + \frac{3}{2} V_{\text{omax}} \left(\frac{[\text{O}_2]}{[\text{O}_2] + K_{\text{o}}} \right) \left(\frac{[\text{RuBP}]}{[\text{RuBP}] + K_{\text{r}}} \right) \\ & + k_2 [\text{PGA2}] [\text{P1}] - k_3 [\text{PGA1}] [\text{ATP1}] \end{aligned} \quad (35)$$

$$\begin{aligned} \frac{d[\text{RuBP}]}{dt} = & k_{10} [\text{DHAP1}] [\text{ATP1}] \\ & - V_{\text{cmax}} \left(\frac{[\text{CO}_2]}{[\text{CO}_2] + K_{\text{c}}} \right) \left(\frac{[\text{RuBP}]}{[\text{RuBP}] + K_{\text{r}}} \right) \\ & - V_{\text{omax}} \left(\frac{[\text{O}_2]}{[\text{O}_2] + K_{\text{o}}} \right) \left(\frac{[\text{RuBP}]}{[\text{RuBP}] + K_{\text{o}}} \right). \end{aligned} \quad (36)$$

Note that in equation (35), the first term on the left hand side is multiplied by a factor of two. This is consistent with the stoichiometry of equation (7), in which one mole of RuBP is oxidized by one mole of CO_2 to yield 2 moles of PGA. In the oxygenase reaction, the factor is 3/2. It remains to consider the pools of CO_2 and O_2 in the stromal compartment [Hall and Bjorkman, 1975; Bruin et al., 1970].

CO_2 and O_2 Concentration in the Stroma

Ambient levels of CO_2 are unaffected by the flux to an individual leaf and can be treated as an unlimited source, while the carboxylation reaction in the leaf's chloroplast acts as a sink. The effect is to create a concentration gradient between the ambient source and each chloroplastic tank. Between source and sink, there are resistances, some of which are more constant than others. The primary resistance to CO_2 flux in terrestrial C_3 plants such as soybeans, is caused by the stomates, which respond to a variety of factors, such as light, leaf water potential and CO_2 concentration. For stomatal response in this model, water stress is ignored, light level is treated as an "opening" switch, and CO_2 concentration does not affect resistance. Under these assumptions, the daytime cellular CO_2 concentration, $[\text{CO}_2]_i$, is treated as a constant for any given ambient CO_2 level [Wong et al., 1979]. The diffusion of CO_2 from the intercellular space, through the cell wall, through the cytoplasm, through the chloroplastic membrane and into the stroma, is the process of interest. In some previous models, this process has been approximated by assuming a CO_2 concentration of zero at the active site [Chartier and Prioul, 1976], and solving for a "mesophyll resistance," which is a conglomerate of various membrane, photorespiration and carboxylation effects. In this model, the reduction

of CO_2 is assumed to take place in the well mixed fluid compartment of the chloroplast, the stroma, which has an average CO_2 concentration $[\text{CO}_2]$. The resistance to diffusive transport between the intercellular space and the stroma is the sum of the two membranes and the intervening cytoplasmic fluid, which is defined as membrane resistance, R_m . This process is modelled as simple diffusion transport, using the equation

$$\text{CER} = \frac{[\text{CO}_2]_i - [\text{CO}_2]}{R_m} \quad (37)$$

Using estimated values for R_m [Charles-Edwards and Ludwig, 1974], $[\text{CO}_2]$ can be defined in terms of CER, which is the carbon dioxide exchange rate.

A second source of stromal CO_2 is the oxidation of phosphoglycolate (PGly) shown in Figure (11). According to work done by Bruin et al. [1970], one mole of oxygenated RuBP produces $3/2$ moles of PGA and $1/2$ mole of CO_2 . The input to the stromal CO_2 tank due to photorespiration can be written as a fraction of equation (34). The rate of export from the CO_2 tank is equal to the carboxylation rate in equation (33). Using these equations as well as equation (37), a differential balance for the stromal CO_2 concentration can be written as follows:

$$\begin{aligned} \frac{d[\text{CO}_2]}{dt} = & \frac{[\text{CO}_2]_i - [\text{CO}_2]}{R_m} \\ & + \frac{1}{2} V_{\text{ormax}} \left(\frac{[\text{O}_2]}{[\text{O}_2] + K_o} \right) \left(\frac{[\text{RuBP}]}{[\text{RuBP}] + K_r} \right) \\ & - 1 V_{\text{cmax}} \left(\frac{[\text{CO}_2]}{[\text{CO}_2] + K_c} \right) \left(\frac{[\text{RuBP}]}{[\text{RuBP}] + K_r} \right). \end{aligned} \quad (38)$$

The oxygen concentration at the reaction site within the chloroplast is a function of diffusion from an unlimited ambient reservoir where

its absolute concentration is 700 times greater than $[CO_2]$. In addition, O_2 is being supplied in the thylakoids, where the light reaction is splitting water faster than the oxygenase reaction can remove it. Deviations from equilibrium with air levels of oxygen caused by the last two processes are assumed to be damped out by the diffusion process. Oxygen concentration is assumed to be in constant equilibrium with ambient levels (21% at 1 atmosphere) [Sinclair et al., 1977].

Membrane Transport Considerations

During analysis of model results, a two-substrate Michaelis-Menten function describing the exchange of DHAP1 for P2 was found to correspond to empirical results much better than a first order function (see Figure 21). Therefore, the term

$$k_5 [DHAP1] [P2]$$

used in equations (14) and (16) has been replaced by the following:

$$V_{dpmax} \left(\frac{[DHAP1]}{[DHAP1] + K_d} \right) \left(\frac{[P2]}{[P2] + K_p} \right).$$

There is ample theoretical basis for modelling membrane transport with a Michaelis-Menten function [Epstein and Hagen, 1952].

A second assumption has been made simply for the sake of convenience. The modelled exchange of P1 for PGA2 has been found to be a relatively small term on a functional basis. Further, direct measurements of this particular process have not been made. Thus, with no direct data on process rate or how it may be altered by chloroplastic or cytoplasmic conditions, the term is too arbitrary to remain in the model. The term

$$k_2 [PGA2] [P1]$$

is assumed to be small and without recognized control significance; therefore it has been dropped from equations (13), (17), (22), and (25).

Competitive Inhibition of Sucrose Formation

As with membrane transport, the formation of sucrose was not well modelled as a first order reaction. During model analysis it was found that sucrose formation could be realistically modelled as a Michaelis-Menten function of [DHAP2] competitively inhibited by [SUCROSE]. Therefore, the term

$$k_8 [\text{DHAP2}] [\text{ATP2}]/[\text{SUCROSE}]$$

used in equations (16) and (18) has been replaced by the following:

$$\frac{V_{d\text{max}} [\text{DHAP2}]}{[\text{DHAP2}] + K_{d2} (1 + [\text{SUCROSE}]/K_s)} \cdot$$

The basis for modelling sucrose formation this way is provided by the results obtained by Hawker [1967]. Those results indicated that the conversion of sucrose phosphate to sucrose is regulated by competitive inhibition of the mediating enzyme by sucrose itself. Since information on [ATP2] is so limited, it has not been included in the new term.

The modelled system of equations as altered by the assumptions in the preceding three sections is listed in Table (4). A final schematic representation of the model as described by the system of equations is given in Figure (12). In addition, a listing of major assumptions involved in the model's development are listed in Table (5).

During model development many explicit and implicit assumptions were made. In the following postscripts two important central assumptions are considered in detail. The first is an explicit assumption about

Table 4. Biochemical photosynthesis model equations.

$$\begin{aligned} \frac{d[\text{PGA1}]}{dt} = & 2 V_{\text{cmax}} \left(\frac{[\text{CO}_2]}{[\text{CO}_2] + K_{\text{c}}} \right) \left(\frac{[\text{RuBP}]}{[\text{RuBP}] + K_{\text{r}}} \right) \\ & + \frac{3}{2} V_{\text{omax}} \left(\frac{[\text{O}_2]}{[\text{O}_2] + K_{\text{o}}} \right) \left(\frac{[\text{RuBP}]}{[\text{RuBP}] + K_{\text{r}}} \right) \\ & - k_3 [\text{PGA1}] [\text{ATP1}] \end{aligned} \quad (39)$$

$$\begin{aligned} \frac{d[\text{DHAP1}]}{dt} = & k_3 [\text{PGA1}] [\text{ATP1}] + 2k_4 [\text{STARCH}] [\text{P1}] \\ & - k_{10} [\text{DHAP1}] [\text{ATP1}] - k_6 [\text{DHAP1}] [\text{PGA1}] [\text{ATP1}]/[\text{P1}] \\ & - V_{\text{dpmax}} \left(\frac{[\text{DHAP1}]}{[\text{DHAP1}] + K_{\text{d}}} \right) \left(\frac{[\text{P2}]}{[\text{P2}] + K_{\text{p}}} \right) \end{aligned} \quad (40)$$

$$\begin{aligned} \frac{d[\text{STARCH}]}{dt} = & \frac{1}{2} k_6 [\text{DHAP1}] [\text{PGA1}] [\text{ATP1}]/[\text{P1}] \\ & - k_4 [\text{STARCH}] [\text{P1}] \end{aligned} \quad (41)$$

$$\begin{aligned} \frac{d[\text{DHAP2}]}{dt} = & V_{\text{dpmax}} \left(\frac{[\text{DHAP1}]}{[\text{DHAP1}] + K_{\text{d}}} \right) \left(\frac{[\text{P2}]}{[\text{P2}] + K_{\text{p}}} \right) \\ & - k_7 [\text{DHAP2}] [\text{ADP2}] \\ & - \frac{V_{\text{dsmax}} [\text{DHAP2}]}{[\text{DHAP2}] + K_{\text{d2}} (1 + [\text{SUCROSE}]/K_{\text{s}})} \end{aligned} \quad (42)$$

$$\frac{d[\text{PGA2}]}{dt} = k_7 [\text{DHAP2}] [\text{ADP2}] \quad (43)$$

$$\begin{aligned} \frac{d[\text{SUCROSE}]}{dt} = & \frac{\frac{1}{4} V_{\text{dsmax}} [\text{DHAP2}]}{[\text{DHAP2}] + K_{\text{d2}} (1 + [\text{SUCROSE}]/K_{\text{s}})} \\ & - [\text{EXPORT}] \end{aligned}$$

Table 4. (contd.)

$$\frac{d[\text{RuBP}]}{dt} = \frac{3}{5}k_{10} [\text{DHAP1}] [\text{ATP1}] \quad (36)$$

$$- V_{\text{crmax}} \left(\frac{[\text{CO}_2]}{[\text{CO}_2] + K_c} \right) \left(\frac{[\text{RuBP}]}{[\text{RuBP}] + K_r} \right) \\ - V_{\text{ormax}} \left(\frac{[\text{O}_2]}{[\text{O}_2] + K_o} \right) \left(\frac{[\text{RuBP}]}{[\text{RuBP}] + K_r} \right)$$

$$\frac{d[\text{ATP1}]}{dt} = k_9 [\text{LIGHT}] [\text{ADP1}] [\text{P1}] \quad (20)$$

$$- k_3 [\text{PGA1}] [\text{ATP1}] - k_4 [\text{STARCH}] [\text{P1}]$$

$$- k_6 [\text{DHAP1}] [\text{PGA1}] [\text{ATP1}]/[\text{P1}]$$

$$- k_{10} [\text{DHAP1}] [\text{ATP1}]$$

$$\frac{d[\text{ADP1}]}{dt} = - \frac{d[\text{ATP1}]}{dt} \quad (21)$$

$$\frac{d[\text{P1}]}{dt} = V_{\text{dpmax}} \left(\frac{[\text{DHAP1}]}{[\text{DHAP1}] + K_d} \right) \left(\frac{[\text{P2}]}{[\text{P2}] + K_p} \right) \quad (44)$$

$$+ 2k_6 [\text{DHAP1}] [\text{PGA1}] [\text{ATP1}]/[\text{P1}]$$

$$- k_9 [\text{LIGHT}] [\text{ADP1}] [\text{P1}] - k_4 [\text{STARCH}] [\text{P1}]$$

$$\frac{d[\text{ATP2}]}{dt} = k_7 [\text{DHAP2}] [\text{ADP2}] \quad (45)$$

$$- \frac{V_{\text{dsmax}} [\text{DHAP2}]}{[\text{DHAP2}] + K_{d2} (1 + [\text{SUCROSE}]/K_s)}$$

$$\frac{d[\text{ADP2}]}{dt} = - \frac{d[\text{ATP2}]}{dt} \quad (24)$$

Table 4. (contd.)

$$\frac{d[P2]}{dt} = \frac{2 V_{dsmax} [DHAP2]}{[DHAP2] + K_{d2} (1 + [SUCROSE]/K_s)} \quad (46)$$

$$- V_{dpmax} \left(\frac{[DHAP1]}{[DHAP1] + K_d} \right) \left(\frac{[P2]}{[P2] + K_p} \right)$$

$$\frac{d[CO_2]}{dt} = ([CO_2]_i - [CO_2])/R_m \quad (38)$$

$$+ \frac{1}{2} V_{ormax} \left(\frac{[O_2]}{[O_2] + K_o} \right) \left(\frac{[RuBP]}{[RuBP] + K_r} \right)$$

$$- V_{crmax} \left(\frac{[CO_2]}{[CO_2] + K_c} \right) \left(\frac{[RuBP]}{[RuBP] + K_r} \right)$$

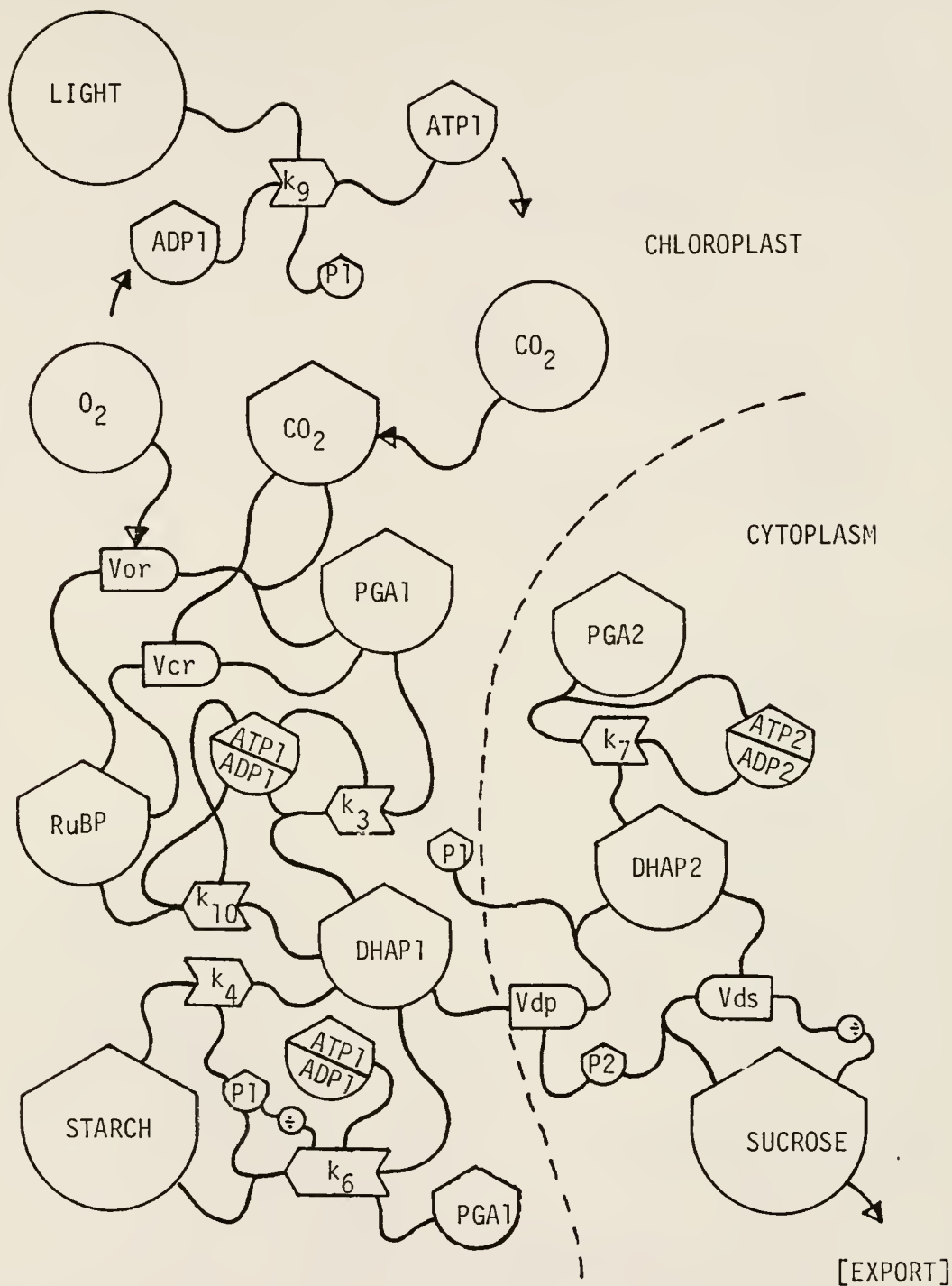


Figure 12. Final photosynthesis schematic. This is a schematic representation of the equations in Table (4) and the assumptions in Table (5). The new rounded interaction symbol indicates a Michaelis-Menten response. All abbreviations are defined in Appendix 1.

Table 5. Key assumptions used in model development.

-
1. Intermediate biochemical compounds that are not at path selecting points and do not allosterically affect enzyme activity levels can be ignored.
 2. RuBP carboxylase-oxygenase is assumed to have a constant activity level which can limit overall CO_2 fixation rate.
 3. Starch and sucrose formation pathways are regulated by allosteric control of enzyme activity levels which can be functionally expressed as concentrations of inhibitors and enhancers.
 4. Concentration of CO_2 in the intercellular space is a constant function of external CO_2 concentration.
 5. Concentration of O_2 in the chloroplast is assumed to be in equilibrium with ambient O_2 level which is constant at a partial pressure of 210 mbar.
 6. The dark reactions all take place in the stroma of the chloroplast which is treated as a well-mixed reaction tank with uniform concentrations throughout.
-

the enzyme kinetics of RuBPc-o and the second is an implicit assumption about the thermodynamically uphill nature of the Calvin cycle. These discussions help to demonstrate the ever-present perils of modelling as well as the insights to be gained from modelling.

Postscript on RuBP Carboxylase-Oxygenase and RuBP Concentrations

Enzyme kinetic theory which was used in the last section to derive the Michaelis-Menten style relationships for photosynthesis and photorespiration relies implicitly on certain assumptions about the behavior of an enzyme.

In the carboxylase reaction described in equation (32), it was assumed that RuBPc had a constant activity level over the range of circumstances for which the analysis applies. More precisely, the well-stirred reaction tank (stroma) is assumed to have a fixed quantity of enzyme, which is either tied up as an enzyme-substrate complex, or is available for reaction. The available portion is expected to have a certain affinity for the various substrates which is unaffected by conditions inside the reaction medium. Recent work suggests that these assumptions may not be applicable to RuBPc-o.

It is well established that the activity level of RuBPc-o, measured in vitro, is highly variable [Bassham et al., 1978]. Until recently, these measurements gave Michaelis-Menten constants which were consistently too high to account for associated photosynthetic rates. It has now been established that, if the enzyme is preincubated in the presence of CO₂ and Mg⁺⁺, then the activity levels in vivo reaction rates can be obtained

[Bahr and Jensen, 1978]. Even under these circumstances, the high activity rate can only be maintained for a short period of time.

Based on their work, Jensen et al. [1978], have suggested that the enzyme exists within the intact chloroplasts in a range of active states which are regulated by the pH and Mg^{++} ion concentration. The chloroplast can be divided into two compartments, the stroma and thylakoid. When exposed to light, a proton gradient is established between these compartments which raises the pH in the stroma. In counter flow to the hydrogen ions moving out, Mg^{++} ions move from the thylakoid to the stroma. Thus, during the day, the enzyme's activity is enhanced and in the dark it is deactivated. In support of this hypothesis, Bassham et al. [1978] found that a stable pool of RuBP is maintained by chloroplast in the dark. This is surprising in view of the extremely negative standard free energy change associated with the carboxylation reaction ($\Delta G^\circ \approx -8.4$ kcal/mole). If the enzyme were active, the reaction would certainly proceed rapidly and irreversibly. Although this evidence seems compelling, very recent work by Robinson et al. [1979] indicates that, in vivo, the activity level changes only slightly. Clearly, the issue is controversial.

This raises the question of whether or not the postulated dependence of photosynthesis on the concentrations of CO_2 , RuBP and O_2 is only partially true. Does the changing pH of the reaction medium alter the enzyme's turnover rate enough to affect the photosynthetic light response curve? Do the assumptions put forth in the various biochemical-empirical models actually predict biochemical observations? An example may clarify the situation.

For a C_3 plant under ambient levels of CO_2 (~ 320 vpm), Wong et al. [1979] have found that, regardless of light level, the intercellular level of CO_2 is constant (~ 250 vpm). Also, assume that R_m which is the CO_2 diffusion resistance associated with cell walls, membranes, cytoplasm and chloroplast stroma, is between 1 and 5 sec/cm [Tenhunen et al., 1977]. Then, rewriting equation (37), the stromal concentration of CO_2 can be solved for any given carbon dioxide exchange rate, CER.

$$[CO_2] = [CO_2]_i - CER * R_m \quad (44)$$

Let $CER1 = 1$ g/m²/hr,

$CER2 = 5$ g/m²/hr and

$R_m = 2$ sec/cm.

Then $[CO_2]_1 = .28$ g CO_2 /m³ ~ 192 vpm

$[CO_2]_2 = .06$ g CO_2 /m³ ~ 41 vpm.

The estimated stromal CO_2 concentration decreases by a factor of 4.67 for a five fold increase in the carbon exchange rate (CER). Assume that the carboxylation reaction rate, V_{cr} , changes in the same proportion as CER, then using standard chemical kinetics to estimate the relative concentrations of RuBP

$$\frac{[RuBP]_1}{[RuBP]_2} = \frac{V_{cr1}}{V_{cr2}} \cdot \frac{[CO_2]_2}{[CO_2]_1} \quad (45)$$

The calculation yields 47.9 times more RuBP in case 2 than in case 1. Using enzyme kinetics as in equation (33) would yield an even larger ratio.

This analysis seems reasonable, at saturating light levels CO_2 becomes limiting while ATP and $NADP_{red}$ levels are high, allowing for

very rapid replacement of RuBP used, thus RuBP levels are high while CO_2 levels are low and vice versa. This conclusion is completely inconsistent with the biochemical literature. Jensen et al. [1978] found that the RuBP pool sizes in isolated chloroplasts were approximately the same under 25 $\mu\text{E}/\text{m}^2/\text{sec}$ and 800 $\mu\text{E}/\text{m}^2/\text{sec}$, even though the carbon exchange rate was 5 times higher under high light. Bassham et al. [1978] found that the RuBP pool size did increase with decreasing $[\text{CO}_2]$ in reconstituted spinach chloroplasts. $[\text{RuBP}]$ concentration increased by 1/3 when CO_2 was decreased from 202 vpm to 116 vpm. Neither of these experiments were done on whole leaves, but the difference in results observed and predicted with the equations above is striking.

At present, the mechanistic nature of the enzyme RuBPC-o is not well enough understood to be included in the model. When sufficient data are available, it can be included by functionally adjusting K_c , K_r , K_o , V_{max} and V_{ormax} to light level, or any other relevant parameter. In the meantime, this exercise will illustrate the potential of modelling biochemical pathways of photosynthesis, but unresolved complexities should serve as a reminder of the limitations of this model.

Postscript on Photosynthesis-Respiration Roles of Chloroplastic PGA

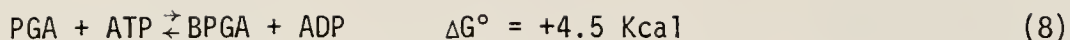
Control over the photosynthetic rate may be directly related to the dual purposes served by the first few reactions of the Calvin cycle. The reversible nature of these reactions was briefly discussed in applying simplifying assumptions to equations (16). The direction of these processes is a function of straight forward thermodynamic considerations. In this model, the direction of this sequence of chemical reactions is not in question, since "forward" reactions predominate during photosynthesis.

However, the thermodynamic requirements that determine the net direction of a reaction are manifested in the concentrations of the reactants. The resulting dynamic balance among the various biochemical intermediates has been postulated to be a central control mechanism regulating photosynthetic rate [Walker and Robinson, 1978]. The following discussion describes the thermodynamics involved and demonstrates how equation (8) is affected.

The first product of photosynthetic CO_2 reduction is 3-phosphoglyceric acid (PGA), which, through three subsequent steps yields DHAP at the expense of one unit each of ATP and NADP_{red} . This process is shown schematically in Figure (5) and the specific equations are numbers 1 through 4 in Table (1). The requirement for light generated energy units in this sequence implies that the pathway is "uphill." Recognizing that the reactions are reversible and that the reverse "downhill" reactions form part of the oxidative glycolytic pathway [Kelly et al., 1976], the situation becomes even more intriguing.

During the daytime, when absorbed light energy is abundant, the chloroplasts are autotrophic, photosynthetically forming energy rich carbohydrates, some of which are exported to the heterotrophic cytoplasm and some of which are stored in the chloroplasts as starch. In the dark, the chloroplasts, like the rest of the cell, are heterotrophic and must obtain energy by breaking down carbohydrates. Thus, in the light the "uphill" energy pathways predominate, while at night, the "downhill" energy releasing paths are activated. PGA and DHAP are both intermediates in both the "uphill" Calvin cycle and the "downhill" glycolytic pathway.

The choice of which pathway is active can be shown by rewriting equation (8):



For a reaction to occur, the free energy change (ΔG) must be negative. Such reactions are termed exergonic. Chemical reactions with a positive free energy change are termed endergonic and will not occur without energy input. Qualitatively, the free energy change is the fraction of total energy change which is available to do work as the system proceeds toward equilibrium, in accord with the second law of thermodynamics. For this reaction, the free energy change (ΔG) is a function of the law of chemical equilibrium (or the mass action law) which simply states that in a system at chemical equilibrium, the concentrations of reactants and products will be such that the following expression holds:

$$K_{eq} = \frac{[\text{BPGA}][\text{ADP}]}{[\text{PGA}][\text{ATP}]},$$

where K_{eq} is the equilibrium constant.

Free energy change is related to the above relationship as follows:

$$\Delta G = \Delta G^\circ + RT \ln \frac{[\text{BPGA}][\text{ADP}]}{[\text{PGA}][\text{ATP}]},$$

where R is the universal gas constant, T is absolute temperature and ΔG is the standard free energy change. At equilibrium conditions for a given temperature, free energy is minimized (entropy is maximized), allowing for no further change in free energy ($\Delta G = 0$). Therefore, the standard free energy is expressed as

$$\Delta G^\circ = -RT \ln (K_{eq}).$$

The net reaction direction, therefore, depends on the concentrations of products and reactants at a particular time. Since the standard free

energy for this reaction is a positive 4.5 Kcal, the balance of concentrations must be such that

$$RT \ln \left(\frac{[BPGA][ADP]}{[PGA][ATP]} \right) < -4.5 \text{ Kcal/mole}$$

if the forward reaction is to proceed. At 25° C the required concentration balance can be calculated for the Calvin cycle to predominate; the ratio of reactants to products must be

$$\left(\frac{[PGA][ATP]}{[BPGA][ADP]} \right) > 2000$$

The ratio ATP/ADP is reported to be in the range of 1 to 10 [Heber, 1974]; therefore, the ratio of PGA to BPGA is expected to range between 2000 and 200. Thus, the chloroplastic levels of BPGA under lighted conditions are expected to be very low [Walker and Robinson, 1978]. This conclusion is consistent with the relative abundance of data on the PGA concentration and the almost total lack of data on BPGA levels.

In summary, when chloroplasts are exposed to light, the Calvin cycle pathway providing RuBP for CO₂ reduction is enzymatically activated, rapidly leading to production of PGA. As the concentration of PGA rapidly increases and the balance of ATP to ADP responds to the balance of light levels and bioenergetic requirements, the "forward reaction" is quickly established and photosynthesis proceeds. Concentrations and flow between tanks quickly reaches a quasi-steady state which is a function of all the factors affecting the balance of intermediates such as light driven regeneration of ATP. These various factors have been incorporated into the model and are analyzed in the following results section.

MODEL RESULTS

The biochemical photosynthesis model outlined in Figure (12) and Table (4) is functionally complete. Given a complete set of data, it would be possible to do computer simulations without further assumptions. However, the data set required would be extensive, precise and subject to wide variations from one set of conditions to another. In an effort to circumvent this problem and to evaluate the model's behavior, a series of simple flow situations have been posed, based on partial data sets and supplemental assumptions. Measured substrate and product fluxes and concentrations of intermediates given in the literature have been used to analyze specific tanks within the model for consistency with the real world.

This section emphasizes the feedback interdependence of the entire system, which expresses itself in control of the photosynthetic rate. At the biochemical level a steady flow system is established in response to external conditions, such that intermediate concentrations are adjusted, partitioning between starch storage and sucrose is delineated and net carbon uptake is fixed. The analysis centers on the flows of CO_2 into and out of the modelled system. The analysis begins with the modelled CO_2 balance, followed by the starch balance, the partition regulating phosphate balances and, finally, the sucrose balance. Working forward along the photosynthetic carbon pathway through each of these key tanks, the response of each balance equation is evaluated. By considering how

the modelled tank balances compare to measured concentrations under differing steady flow situations, the model's usefulness can be tested.

The Chloroplastic Carbon Dioxide Balance, $[CO_2]$

The CO_2 concentration balance in the stroma of the chloroplast is described in the model by equation (38):

$$\begin{aligned} \frac{d[CO_2]}{dt} = & \frac{[CO_2]_i - [CO_2]}{R_m} + \frac{1}{2} V_{o\max} \left(\frac{[O_2]}{[O_2] + K_o} \right) \left(\frac{[RuBP]}{[RuBP] + K_r} \right) \quad (38) \\ & - V_{c\max} \left(\frac{[CO_2]}{[CO_2] + K_c} \right) \left(\frac{[RuBP]}{[RuBP] + K_r} \right), \end{aligned}$$

where the right hand terms represent fluxes due to diffusion, photo-respiration and photosynthesis, respectively. Under steady flow conditions, the chloroplastic CO_2 concentration is in steady state and equation (38) can be written as follows:

$$\begin{aligned} \frac{[CO_2]_i - [CO_2]}{R_m} = & \left(\frac{[RuBP]}{[RuBP] + K_r} \right) \left[\left(\frac{V_{c\max} [CO_2]}{[CO_2] + K_c} \right) - \frac{1}{2} \left(\frac{V_{o\max} [O_2]}{[O_2] + K_o} \right) \right]. \quad (47) \end{aligned}$$

The equation has been rearranged so that each side equals the net flux of ambient CO_2 into the chloroplasts, referred to as net photosynthesis or the carbon dioxide exchange rate (CER). Thus the following two equations can be written as:

$$CER = \frac{[CO_2]_i - [CO_2]}{R_m} \quad (37)$$

$$CER = \left(\frac{[RuBP]}{[RuBP] + K_r} \right) \left[\left(\frac{V_{c\max} [CO_2]}{[CO_2] + K_c} \right) - \frac{1}{2} \left(\frac{V_{o\max} [O_2]}{[O_2] + K_o} \right) \right] \quad (48)$$

Equation (48) involves eight biochemical parameters on the right hand side, all of which have been measured and reported under a variety of circumstances with variable credibility. The parameter values to be used in the following analysis are listed in Table (6). It is useful to recognize parameters in Table (6) as two separate groups. The first group includes K_o , V_{ormax} , K_c , V_{cmax} and K_r , which are assumed constant, although that depends entirely on the constancy of the activity of the enzyme, RuBPc-o. The second group, $[O_2]$, $[CO_2]$ and $[RuBP]$, is assumed to vary, although under real world conditions, $[O_2]$ is approximately constant.

By rearranging equation (48), the values in Table (6) can be used to solve for $[CO_2]$ as follows:

$$[CO_2] = \frac{K_c \left[CER \left(\frac{[RuBP] + K_r}{[RuBP]} \right) + \frac{1}{2} \left(\frac{V_{\text{ormax}} [O_2]}{[O_2] + K_o} \right) \right]}{V_{\text{cmax}} - \left[CER \left(\frac{[RuBP] + K_r}{[RuBP]} \right) + \frac{1}{2} \left(\frac{V_{\text{ormax}} [O_2]}{[O_2] + K_o} \right) \right]}$$

$$= 128 \text{ vpm} .$$

For the specific experimental treatment presented by Heldt et al. [1977], the $[CO_2]$ is 128 vpm or $4.3 \mu\text{M}$ dissolved CO_2 . As a check on the value, equation (47) can be rearranged to solve for R_m .

$$R_m = ([CO_2]_i - [CO_2]) / CER = 1.84 \text{ sec/cm}$$

The value obtained compares well with R_m values found by various independent researchers. Several values are presented in Table (7) for comparison.

Table 6. In vivo biochemical parameters used in model evaluation.

Biochemical Parameter	<u>In Vivo</u> Value	Reference
[CO ₂] _a	320 vpm	Wong et al. [1979]
[CO ₂] _i	230 vpm	Wong et al. [1979]
K _c	230 vpm	Bahr and Jensen [1978]
V _c max	300 μmol CO ₂ /mg chl/hr	Farquahr et al. [1980]
[O ₂]	210 mbar	Sinclair et al. [197]
K _o	330 mbar	Farquahr et al. [1980]
V _o max	80 μmol O ₂ /mg chl/hr	Kent and Young [1980]
[RuBP]	280 μM	Heldt et al. [1978]
K _r	30 μM	Bassham et al. [1978]

Note: This table contains recent approximation-measurements of the various parameters in equations (47) and (48). The term in vivo refers to the functioning living plant; such values are not necessarily constant when biochemical systems are reproduced outside the functioning plant, in vitro. Abbreviations are in Appendix 1.

Table 7. Mesophyll resistance values from the literature.

Mesophyll Resistance	Crop	Reference
6.8 sec/cm	Bean	Chartier et al. [1970]
2.3 sec/cm	Wheat	Ku and Edwards [1977]
2.9 sec/cm	Cotton	Jones and Slayter [1972]

Note: This table contains suggested R_m values for C_3 plants as determined by independent researchers. These values compare favorably with the value obtained using Table (6) in vivo parameters.

As a first analysis of the model sensitivity, a series of CER vs $[CO_2]$ response curves at various constant RuBP concentrations have been generated while $[O_2]$ is constant at 210 mbars. Since both RuBP and CO_2 are substrates in the carboxylation reaction, it is expected that to increase either one will increase photosynthetic rate. As shown in Figure (13), the modelled responses are consistent with reported CER responses to CO_2 concentrations.

In a more specific test of the model's sensitivity, equation (48) is used to generate CER from 6 pairs of RuBP and CO_2 concentrations for comparison with data obtained by Bassham et al. [1978] in experimental work with reconstituted spinach chloroplasts. When the values in Table (6) are used, the results are greatly shifted. Recognizing that RuBP c-o activity is certainly reduced in vitro, values for V_{cmax} and $V_{o_{max}}$ were lowered to $140 \mu\text{mol } CO_2/\text{mg chl/hr}$ and $28 \mu\text{mol } O_2/\text{mg chl/hr}$, and K_c increased to 400 vpm. With the modified biochemical constants, the comparison between modelled and measured values is quite good.

The characteristic behavior of the simulated CER response to $[CO_2]$ is consistent with the in vitro data of Bassham et al. [1978]. In addition, the magnitude of the simulated response was similar to those results. Simulated and measured values are compared in Figure (14).

The effect of photorespiration can be examined by varying the level of O_2 for different fixed values of $[CO_2]$ and $[RuBP]$. Results are graphically presented in Figure (15). The trends predicted for changing O_2 values are consistent with general observations. Higher levels of oxygen increase the rate of photorespiration and decrease the carbon dioxide exchange rate.

The results of the modelled initial fixation of carbon are generally consistent with empirically observed behavior. Moving forward through

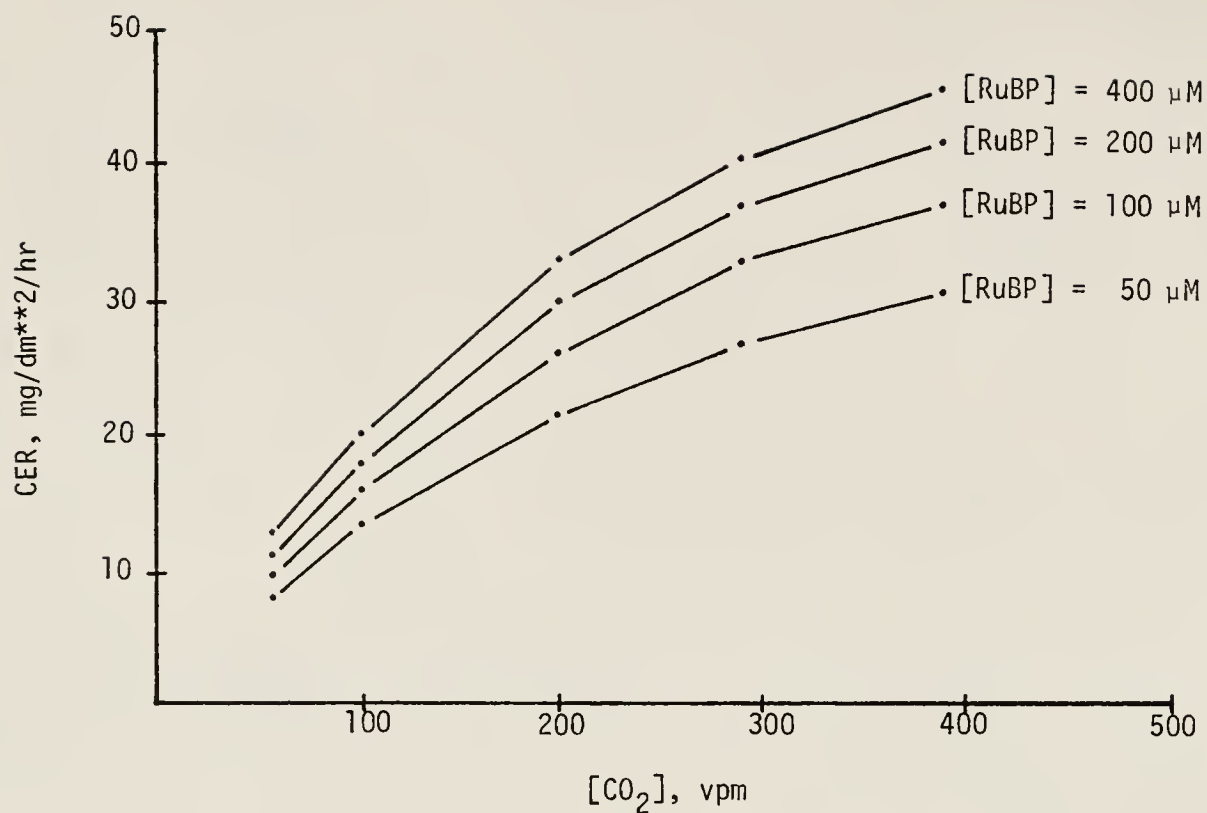


Figure 13. Modelled $[CO_2]$ -photosynthesis- $[RuBP]$ response curves. CER is modelled in units of $mg CO_2$ fixed/dm² leaf area/hour at four different fixed concentrations of RuBP. $[O_2]$ is assumed to be in equilibrium with an atmospheric partial pressure of 210 mbar.

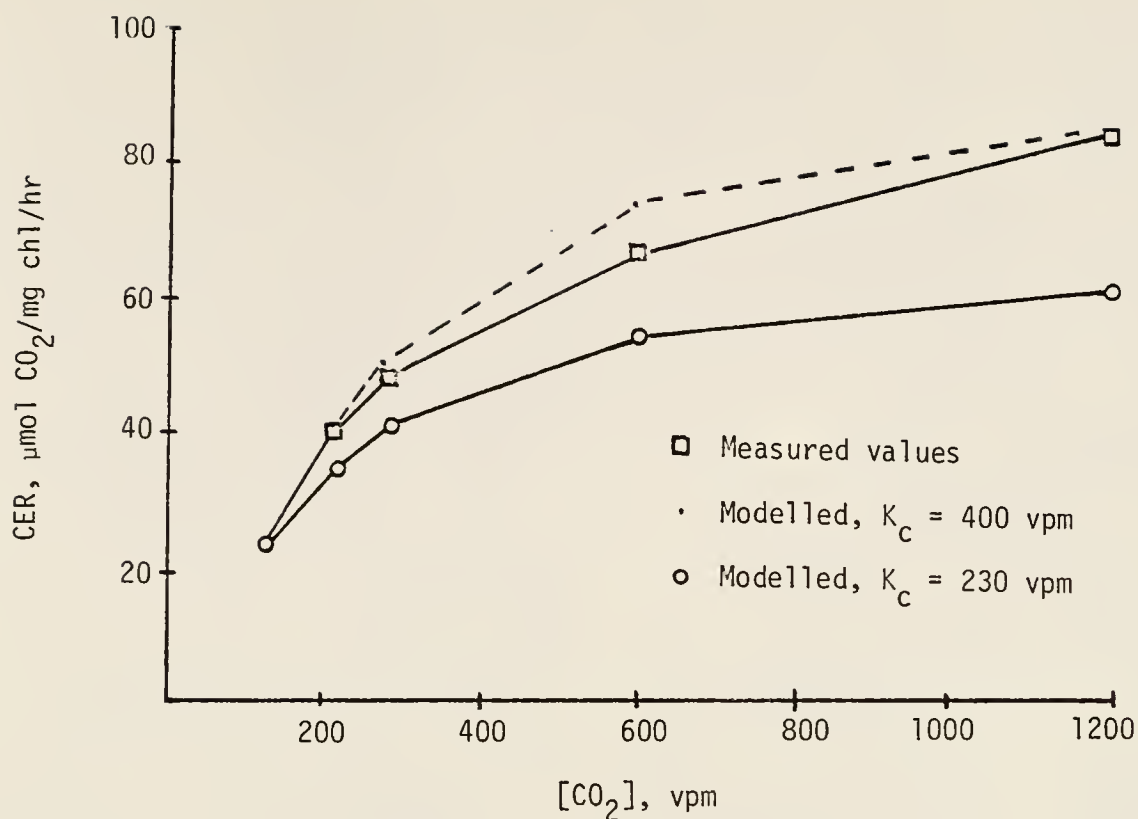


Figure 14. Measured and modelled $[CO_2]$ -photosynthesis response curves. Graphs compare measured values from Bassham et al. [1978] with values from the model. The measured values were obtained in vitro. Therefore, in vitro rate constants listed in Table (8) were used to obtain the model values. The difference between the curves is in the K_c values. For the dashed curve K_c equals 400 vpm. The other curve has a K_c value of 230 vpm.

Table 8. Comparison of in vivo and in vitro biochemical parameters.

Biochemical Parameters	<u>In Vivo</u> Values	<u>In Vitro</u> Values
Kc	230 vpm	400 vpm
Vc _{max}	300 $\mu\text{mol CO}_2/\text{mg chl/hr}$	140 $\mu\text{mol CO}_2/\text{mg chl/hr}$
Ko	330 mbar	330 mbar
V _o _{max}	80 $\mu\text{mol O}_2/\text{mg chl/hr}$	37 $\mu\text{mol O}_2/\text{mg chl/hr}$
Kr	30	30

Note: In vitro values are primarily from Bassham et al. [1978] and in vivo values are from Table (6). All abbreviations are defined in Appendix 1.

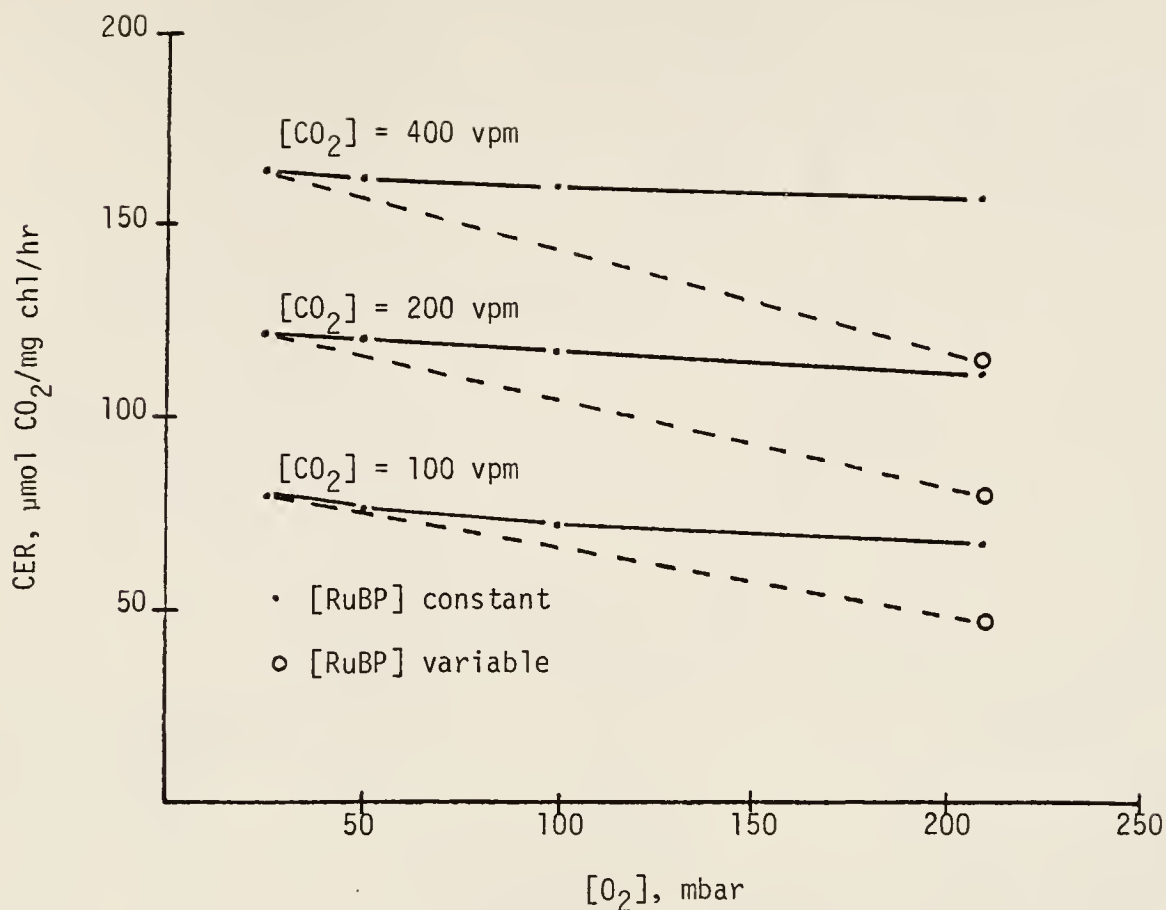


Figure 15. Modelled $[O_2]$ -photosynthesis response curves. For the solid lines $[RuBP]$ is constant at $280 \mu\text{M}$. For the dashed line $[RuBP]$ is $280 \mu\text{M}$ for the left most value and $50 \mu\text{M}$ for the right most value. Since increased $[O_2]$ increases the competition for $RuBP$ its level is expected to decline as in the dashed lines.

the model, fixed carbon is partitioned between paths leading to export and to storage in the starch tank. Although it represents a relatively small fraction of carbon fixed, starch formation rate and concentration are highly visible barometers of shifting biochemical balances within photosynthetic cells. As such, it is the next model compartment for consideration.

Time Rate of Change of Starch

As an initial test of the starch balance predicted by the model in equation (15), fluxes to the starch tank under light and dark conditions have been considered. Since starch has been widely observed to accumulate during the day and to be remobilized in the dark [Heldt et al., [1977], equation (15) should show a net flux into the starch tank under lighted conditions and net export in the dark. Rewriting equation (15)

$$\begin{aligned} \frac{d[\text{STARCH}]}{dt} = & \frac{1}{2}k_6 [\text{DHAP1}] [\text{ATP1}] [\text{PGA1}] / [\text{P1}] \\ & - k_4 [\text{P1}] [\text{STARCH}] , \end{aligned} \quad (15)$$

the first term on the right hand side is the functional rate of starch formation and the second term is the rate of mobilization and export of starch. There are five variables and two constants to be considered in solving for the starch flux, $d[\text{STARCH}]/dt$. Although these variables are dynamic, changing rapidly in response to external conditions, they tend to have fundamentally different values under light and dark conditions. Table (9) lists relative values suggested from the literature for some of the variables.

With these values, the rate of starch formation term would be a factor of 20 lower in the dark than in the light, while the starch

Table 9. Chloroplastic concentration of key metabolites in light and dark.

Metabolite	Light	Dark	Reference
[PGA1]	4.0 mM	1.6 mM	Kaiser and Bassham [1979]
[DHAP1]	.4 mM	.4 mM	Kaiser and Urbach [1977]
[P1]	3.0 mM	12.0 mM	Kaiser and Bassham [1979]
[ATP1]	2.0	1.0	Heber [1974]

Note: Recently measured-approximated concentrations of metabolites important to starch formation and remobilization. The values for ATP1 are relative numbers only.

mobilization term would be 4 times higher in the dark than in the light. Equation (15) predicts that, in the light, starch formation will predominate, while in the dark, starch will be exported. This result is consistent with observed empirical behavior.

If the variables [DHAP1], [ATP1], [PGA1] and [P1] are assumed to be constant, the starch balance can be written as

$$\frac{d[\text{STARCH}]}{dt} = A - B [\text{STARCH}] , \quad (49)$$

where $A = \frac{1}{2}k_6 [\text{ATP1}] [\text{DHAP1}] [\text{PGA1}] / [\text{P1}]$ and $B = k_4 [\text{P1}]$. This equation can be integrated to yield

$$[\text{STARCH}] = A (1 - e^{-Bt})/B , \quad (50)$$

which is valid as long as A and B are constant. Under steady flow conditions, intermediates are at least roughly in steady state. In experimental work done by Upmeyer and Koller [1973], soybean seedlings were grown under constant conditions: saturating light, 300 vpm CO₂, 25° C and 60% relative humidity. Artificial lighting was switched on for 16 hours and off for 8 hours every day. Their data indicated that, from four hours after the lights were switched on until twelve hours later, the CO₂ exchange rate (CER) was constant at 34 mg/dm²/hr. These conditions approximate a steady flow state; therefore, the variables A and B can be assumed constant.

Measurements of starch flux and concentration taken directly from Upmeyer and Koller's data can be plugged into equations (49) and (50) to solve for A and B. Values are given in Table (10).

The predicted values of [STARCH] based on Table (10) values of A and B are plotted graphically with the experimentally measured values in Figure (16).

Table 10. Measured starch accumulation parameters.

Hours Under Constant Light	$\frac{d[\text{STARCH}]}{dt}$	[STARCH]
4	4.25 mg/dm ² /hr	25.5 mg/dm ²
12	.87 mg/dm ² /hr	46.0 mg/dm ²

$$A = 8.45 \text{ mg/dm}^2/\text{hr}$$

$$B = .16 \text{ hr}^{-1}$$

Note: Values are from graphical data presented by Upmeyer and Koller [1973]. These values are used to calculate parameters A and B from equation (49).

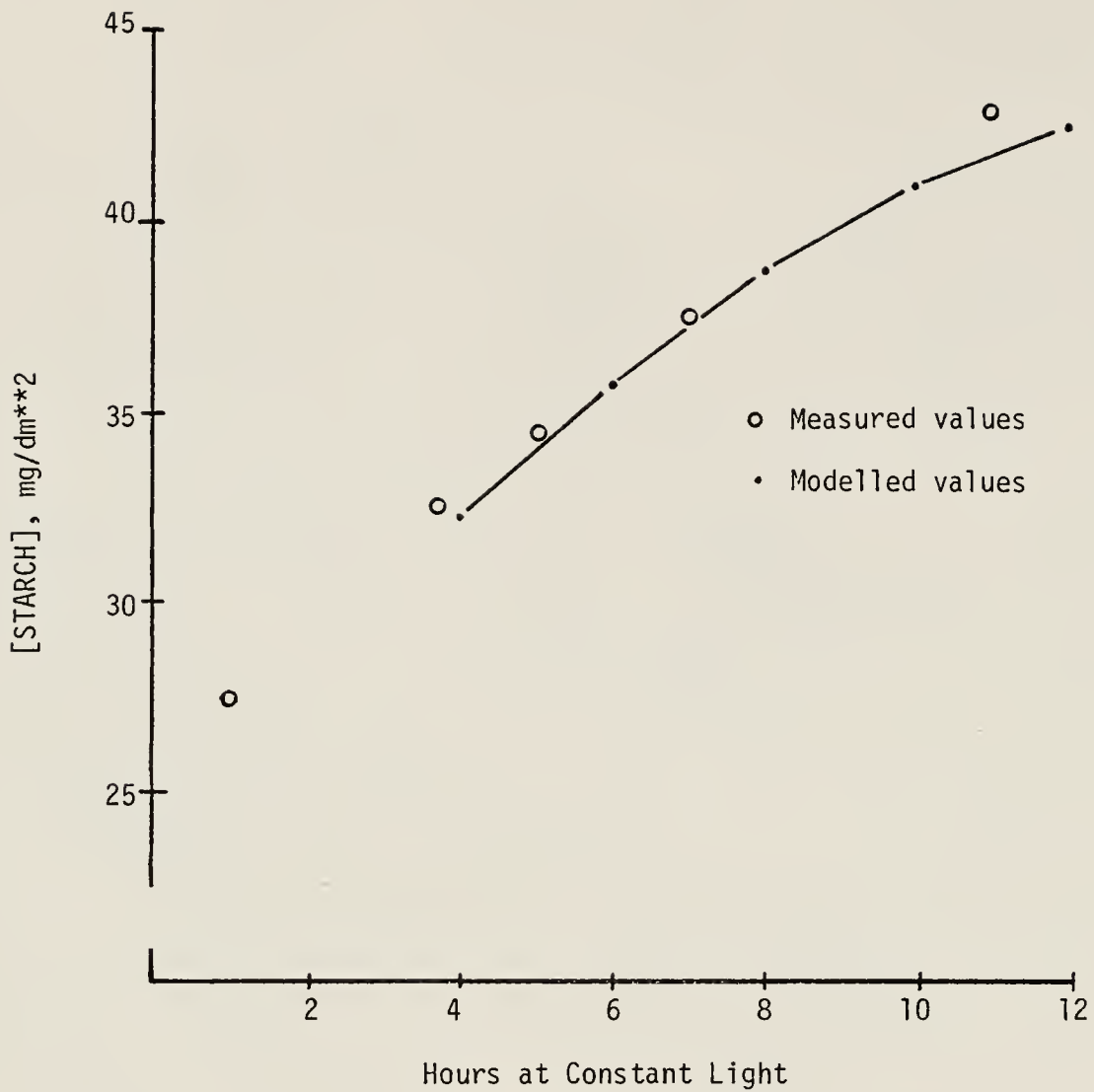


Figure 16. Measured and modelled starch accumulation response. Measured values are from Upmeyer and Koller [1973].

Since the level of chloroplastic inorganic phosphate $[P_i]$ is proportional to starch mobilization and inversely proportional to starch formation, it is the primary functional mechanism affecting the starch accumulation rate. To determine how changes in $[P_i]$ affect $[STARCH]$ a proportionate family of constants was calculated.

The ratio A/B is numerically equivalent to the maximum predicted concentration of starch. The curves resulting from the five sets of constants are given in Figure (17).

The general trend predicted by the set of curves in Figure (17) is consistent with observations that starch accumulation is increased as inorganic phosphate levels are decreased. In experimental work comparing starch levels in phosphate deficient plants and phosphate rich plants, starch concentrations were as much as 10 times higher in the plants deprived of phosphate [Herold et al., 1976]. The starch levels predicted and graphed in Figure (17) mimic these observations, ranging over a factor of approximately 10.

A more realistic analysis of the model's starch balance must consider the other variables $[DHAP_i]$, $[PGA_i]$ and $[ATP_i]$ in addition to inorganic phosphate $[P_i]$. The regulatory role played by the precise mix of these variables can be explored in more detail by using a set of data in which the chloroplastic level of $[P_i]$ was held at four differing quasi-steady state levels. Heldt et al. [1977] manipulated $[P_i]$ levels in a suspension of chloroplasts by controlling the level of inorganic phosphate in the medium external to the chloroplasts. This is equivalent to controlling $[P_2]$ in the model. Experiments were short term, 10 minutes and all external variables such as light, temperature and pre-treatment of chloroplasts were the same for each $[P_2]$. Pertinent data from the experiments are listed in Table (12).

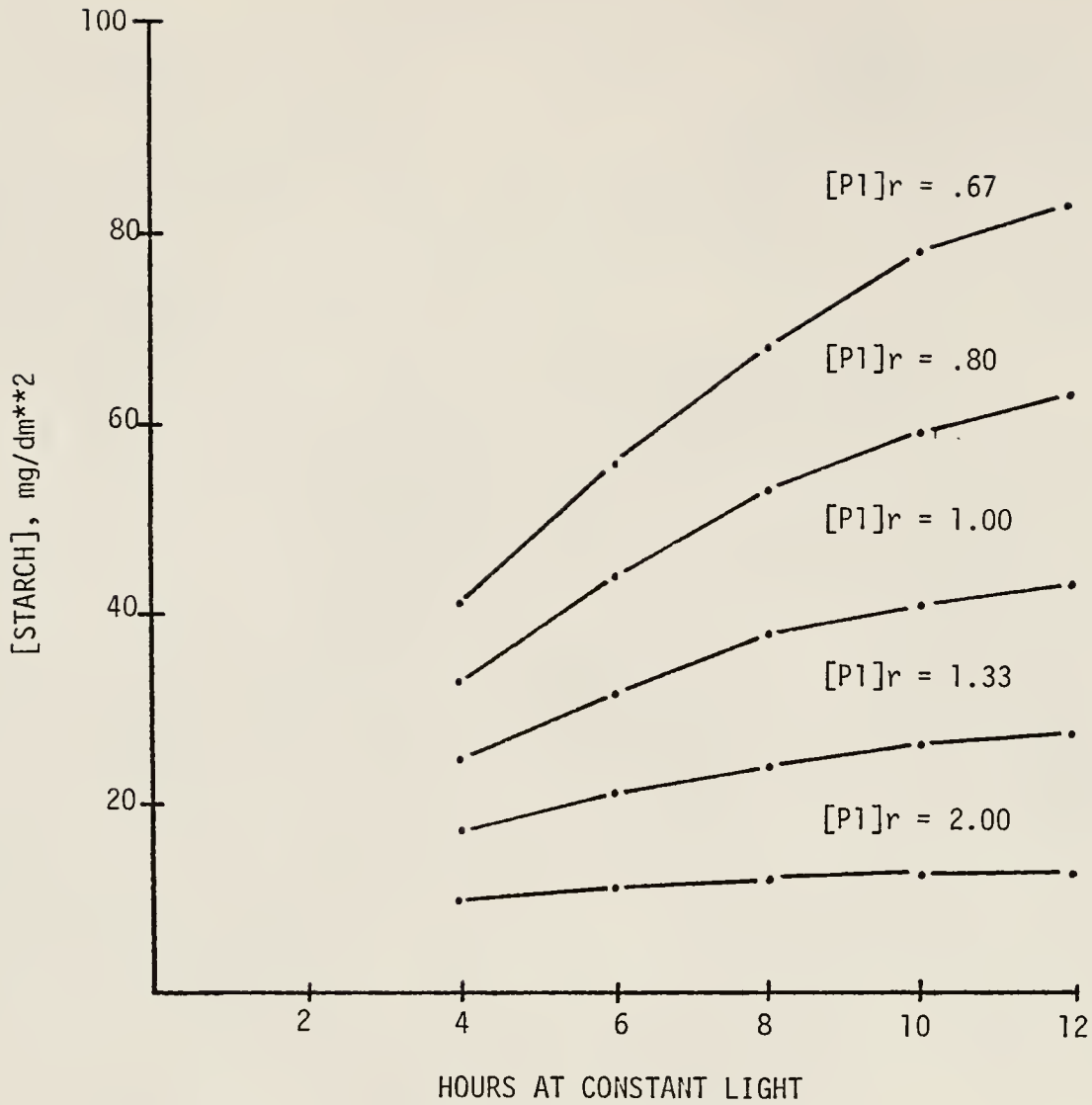


Figure 17. Time courses of modelled inorganic phosphate-starch response. [P1]r is the relative concentration of chloroplastic inorganic phosphate. It is equal to the normalizing ratio $[P1]_n/[P1]_3$ given in Table 11.

Table 11. Starch accumulation as a function of inorganic phosphate concentration.

n	$[P1]_n/[P1]_3$	A_n mg/dm ^{**2} /hr	B_n hr ⁻¹	A_n/B_n mg/dm ^{**2}
1	2.00	4.22	.33	12.8
2	1.33	6.34	.22	28.9
3	1.00	8.45	.16	51.3
4	.80	10.56	.13	80.2
5	.67	12.67	.11	115.5

Note: This table demonstrates how changes in the relative value of $[P1]$ effect the starch formation parameters A and B. Relative values of $[P1]$ are obtained by normalizing $[P1]_n$ by $[P1]_3$.

Table 12. Measured chloroplastic-cytoplasmic inorganic phosphate interactions.

Metabolites Mm	Treatment			
	1	2	3	4
[P2]	.96	.43	.19	.08
[P1]	9.60	7.00	4.00	2.20
[PGA2]	.004	.009	.016	.024
[PGA1]	2.90	6.00	6.90	8.90
[DHAP1]	.17	.33	.40	.25
[RuBP]	3.20	3.70	4.10	4.90
Rates $\mu\text{mol CO}_2/\text{mg chl/hr}$				
[STARCH]	.30	1.40	7.90	7.70
CER	91.3	108.40	113.90	59.70

Note: Metabolite and rate responses to changes in the cytoplasmic level of inorganic phosphate. Values are from Heldt et al. [1977]. No data on ATP levels were given. Abbreviations are in Appendix 1.

Assuming that in this short experiment starch levels are very low, the mobilization term can be neglected. Therefore, starch accumulation is a function only of the starch formation rate:

$$\frac{d[\text{STARCH}]}{dt} = \frac{1}{2}k_6 [\text{ATP1}] [\text{DHAP1}] [\text{PGA1}] / [\text{P1}] . \quad (51)$$

Values predicted by this equation are compared with measured values in Figure (18). (No values for [ATP1] were given, so for convenience of scale, assume that $k_6^* [\text{ATP1}]$ is constant and equals 7.7.)

For the various steady flow situations outlined, the model correctly mimics qualitative behavior and corresponds reasonably well with absolute values. From this analysis, the most obvious feature of the modelled starch tank to emerge is the central regulating role played by chloroplastic levels of inorganic phosphate. To further explore the inorganic phosphate control mechanism as it affects starch accumulation as well as carbon export, the [P1] tank is treated in the following section.

The Chloroplastic Inorganic Phosphate Balance, [P1]

In the experimental work summarized by the data in Table (12), different levels of P1 were maintained by manipulating the inorganic phosphate concentrations in the medium, external to the chloroplasts. This was equivalent to adjusting the cytoplasmic inorganic phosphate concentration, [P2]. The correspondence between [P1] and [P2] is quite strong, as can be seen in Table (12), and graphically in Figure (19).

This strong proportional dependence should be reflected in equation (44), which is the chloroplastic inorganic phosphate balance equation:

$$\begin{aligned} \frac{d[\text{P1}]}{dt} = & \frac{V_{dpmax} [\text{DHAP1}] [\text{P2}]}{([\text{DHAP1}] + K_d)([\text{P2}] + K_{p2})} + 2k_6 [\text{DHAP1}] [\text{PGA1}] [\text{ATP1}] / [\text{P1}] \\ & - k_9 [\text{LIGHT}] [\text{ADP1}] [\text{P1}] - k_4 [\text{STARCH}] [\text{P1}] . \end{aligned} \quad (44)$$

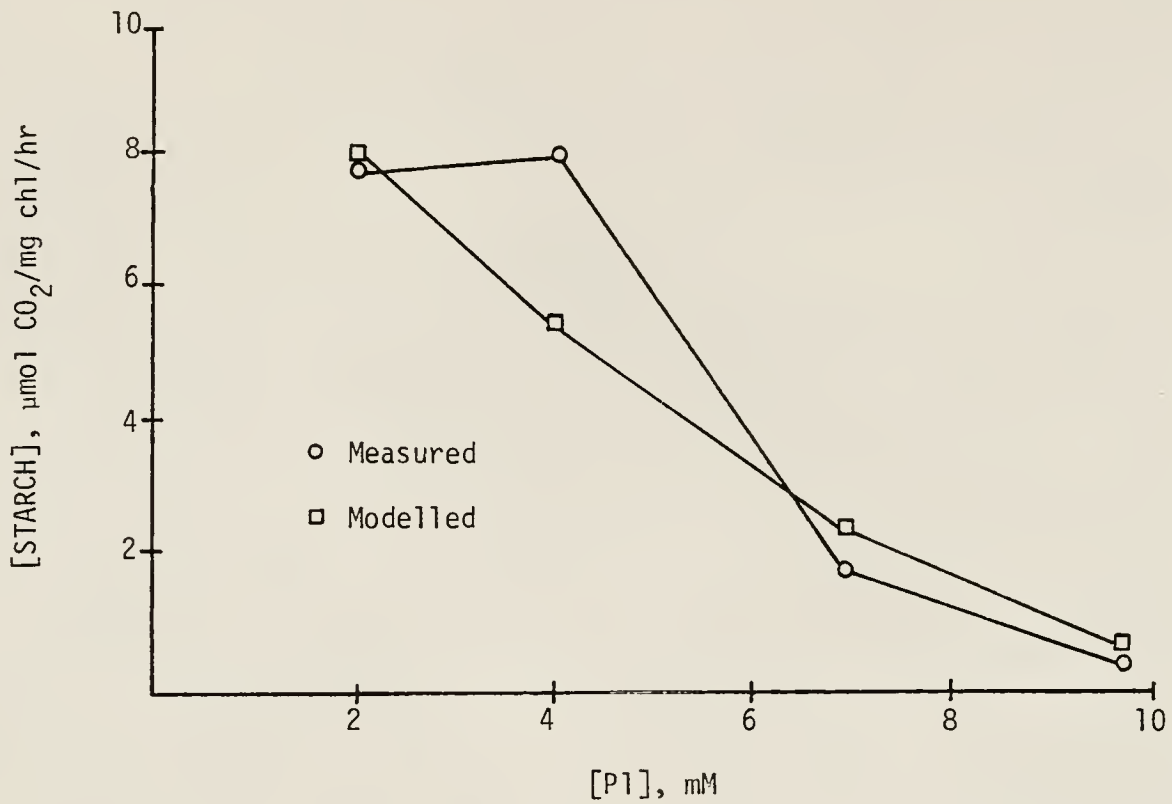


Figure 18. Measured and modelled inorganic phosphate-starch response. Graph compares measured and modelled rates of starch formation as a function of chloroplastic levels of inorganic phosphate, [P1]. Starch accumulation rates are in units of $\mu\text{mol CO}_2$ fixed as starch/mg chlorophyll/hour.

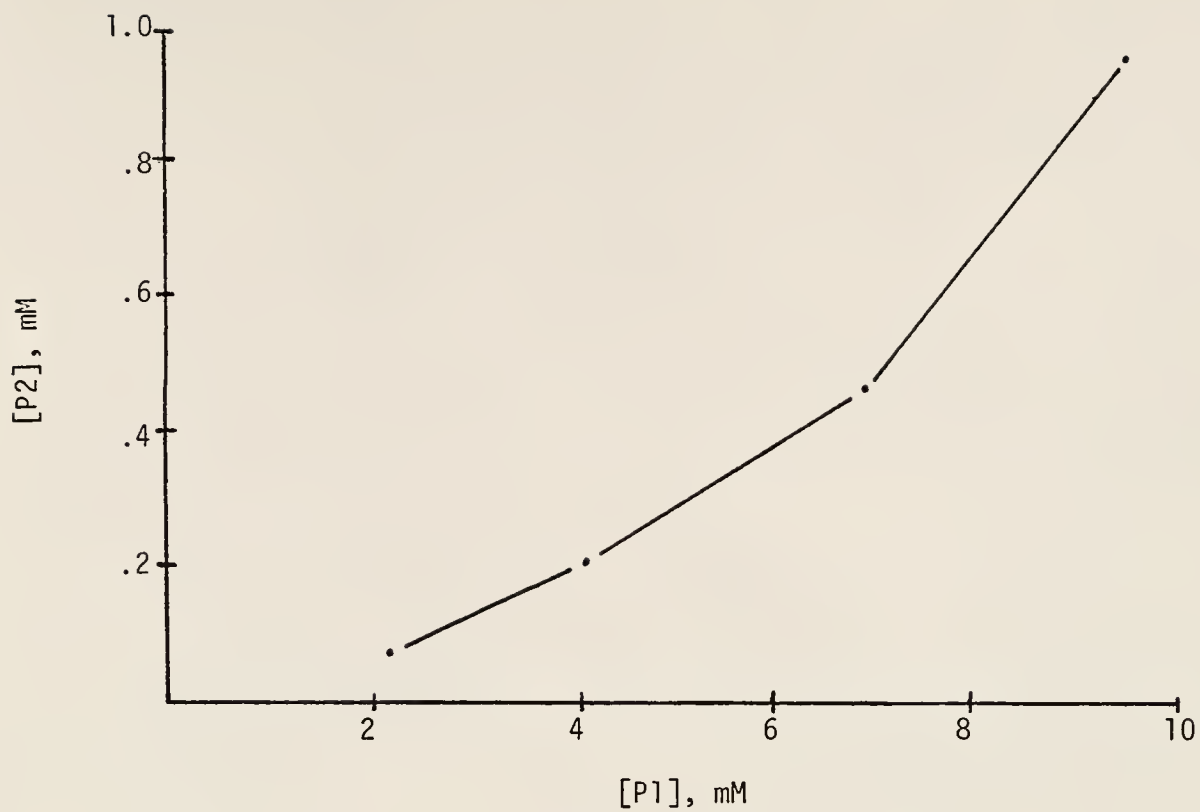


Figure 19. Comparison of chloroplastic and external concentrations of inorganic phosphate. Based on data from Heldt et al. [1977].

In equation (44), the modelled dependence of $[P1]$ for $[P2]$ is in the first term on the right hand side. This term represents the export of DHAP1 from the chloroplast in strict counter exchange for inorganic phosphate from the cytoplasm, $P2$. The second term on the right hand side is the flux to the $P1$ pool, resulting from the net dephosphorylation of glucose phosphate in the accumulation of starch. Comparing the rates of starch accumulation with CO_2 assimilation rates in Table (12), it is clear that much more fixed carbon was being exported (term 1) than was being stored as starch (term 2). This relationship is illustrated graphically in Figure (20). Numerically, in the most extreme case with $[P1]$ equal to 2.2 mM in treatment 4, starch accumulation was approximately 13% of the total carbon dioxide fixed and sucrose export was 87% of CO_2 fixed.

In other experimental work, the maximum rates of starch buildup are cited as being from 10% to 20% under normal conditions [Herold and Walker, 1979]. Based on these data, the first term on the right hand side of equation (44) is the primary process supplying inorganic phosphate to the chloroplast. Therefore, the strong measured functional dependence of $[P1]$ on $[P2]$ is also a predominant feature of the modelled $[P1]$ balance.

In the discussion above, the modelled correspondence between the first term on the right hand side and export of DHAP1 to the sucrose tank are equated. This can be expressed as

$$[TRANSPORT] = V_{dpmax} \left(\frac{[DHAP1]}{[DHAP1] + K_{d1}} \right) \left(\frac{[P2]}{[P2] + K_p} \right), \quad (52)$$

where $[TRANSPORT]$ equals the time rate of DHAP1 transport to the cytoplasm. Equation (52) can be tested directly, using the data in Table (12), along with assumed values for K_{d1} , K_p and V_{dpmax} . Results are shown in Figure (21).

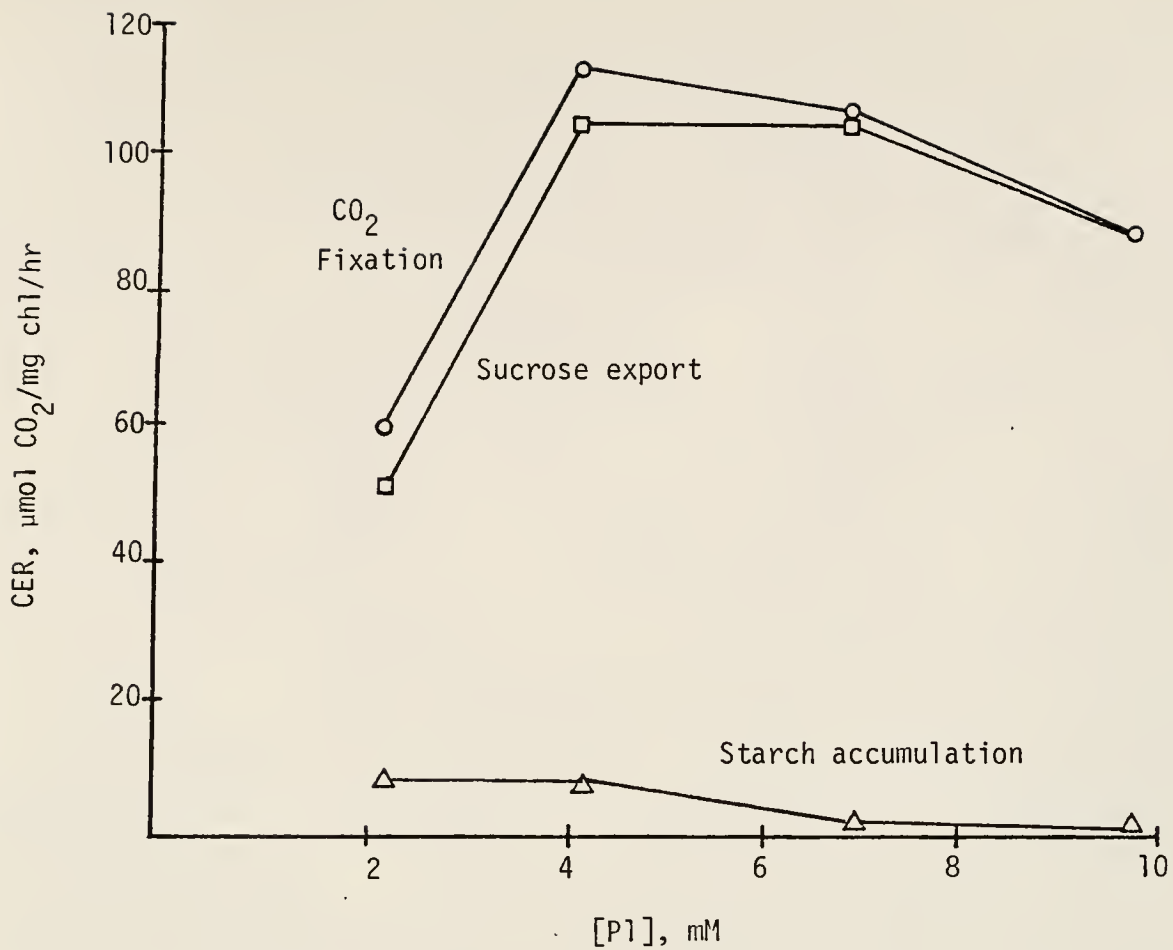


Figure 20. Comparison of carbon partitioning pathways. Compares total CO_2 fixation rate with the rates of sucrose export and starch accumulation at various measured levels of chloroplastic inorganic phosphate, $[P1]$. Based on data from Heldt et al. [1977]. CER is given in units of micromoles CO_2 fixed/mg chlorophyll/hr.

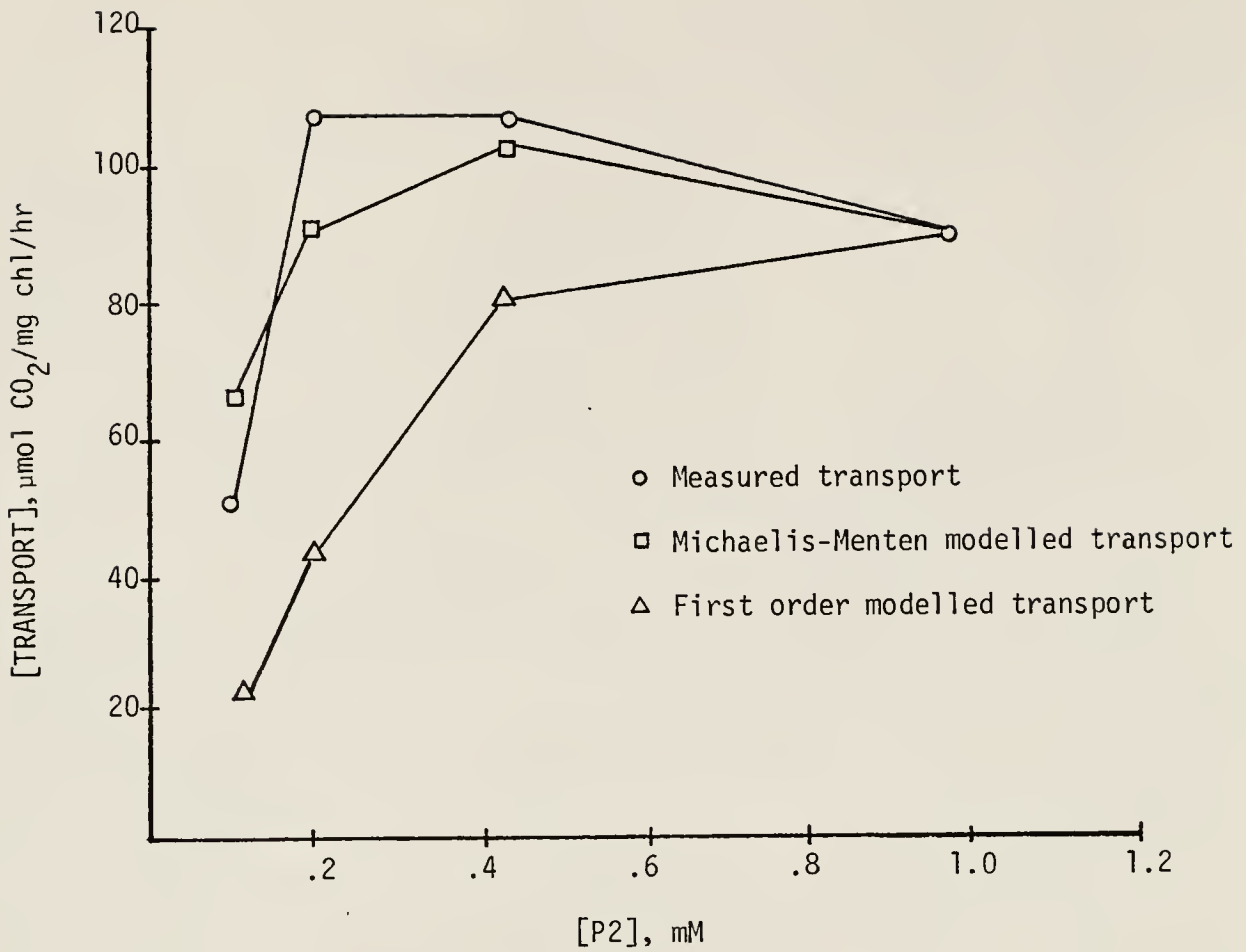


Figure 21. Measured and modelled inorganic phosphate-transport response. Graphs compare the amount of fixed carbon transported from chloroplast to cytoplasm as a function of $[P_2]$. Modelled curve constants were adjusted to intersect at the right most point. Measured values are from Heldt et al. [1977].

For a comparison, a first order relationship is also tested and illustrated in Figure (21). The first order function has the following form:

$$[\text{TRANSPORT}] = K_5 [\text{DHAP1}] [\text{P2}] . \quad (53)$$

The modelled term based on two substrate Michaelis-Menten kinetics clearly fits the observed data better than the first order relationship. Under steady flow conditions, such that CO_2 uptake (CER) equals carbohydrate production over a given period of time, concentrations of the various cyclical intermediates are very nearly in steady state. With this assumption, the [P1] balance can be analyzed in steady state, which can be written as follows:

$$\left(\frac{V_{dpmax} [\text{DHAP1}]}{[\text{DHAP1}] + K_d} \right) \left(\frac{[\text{P2}]}{[\text{P2}] + K_{p2}} \right) + 2(k_6 [\text{DHAP1}] [\text{PGA1}] [\text{ATP1}] / [\text{P1}] - k_4 [\text{STARCH}] [\text{P1}]) = k_9 [\text{LIGHT}] [\text{ADP1}] [\text{P1}] . \quad (54)$$

In equation (54), the first term equals export from the chloroplast to the sucrose tank, the second term is the net accumulation of starch within the chloroplast. Taken together, the terms on the left hand side represent total carbohydrate formation rate which, in steady flow, must equal the carbon dioxide exchange rate. The right hand side of the equation is simply the rate of [ATP1] formation.

Using data from Table (12), equation (54) can be solved for the term $k_9 [\text{LIGHT}] [\text{ADP1}]$ at different values of [P1]. Since [LIGHT] is constant in all four treatments, differences in the term reflect changing values of [ADP1]. Results for the four treatments described in Table (12) are listed in Table (13) and graphed in Figure (22).

The results show an increase in [ADP1] as [P1] decreases. If the sum of [ADP1] and [ATP1] is assumed constant, then [ATP1] levels must decline

Table 13. Adenosine diphosphate formation as a function of inorganic phosphate levels.

Value	Treatment			
	1	2	3	4
[P _i] mM	9.6	7.0	4.0	2.2
CER mol CO ₂ /mg chl/hr	91.3	108.4	113.9	59.7
k _g [LIGHT] [ADP _i]	9.5	15.5	28.5	27.2

Note: This table uses values from Table (12) to evaluate the relative levels of ADP_i that result from changing P_i levels. [LIGHT] and k_g are constant in each treatment.

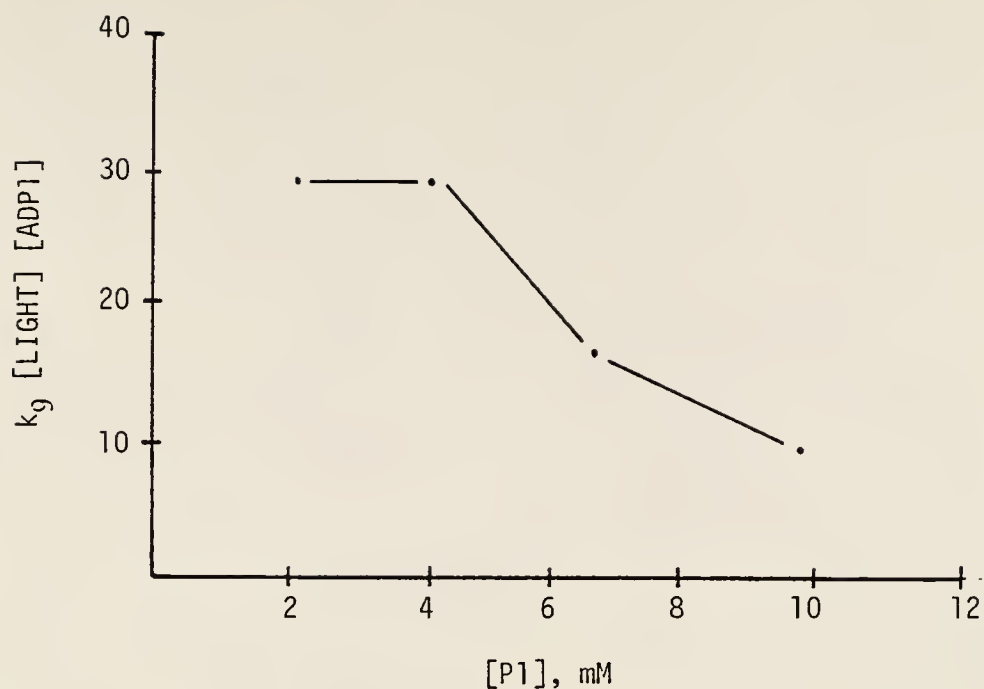


Figure 22. Modelled adenosine diphosphate-inorganic phosphate response. Based on data from Heldt et al. [1977]. [P1] is the chloroplastic concentration of inorganic phosphate. Adenosine diphosphate is a function of [LIGHT] which was constant in the experimental work and the rate constant, k_g . Therefore, changes in the $k_g [\text{LIGHT}] [\text{ADP1}]$ term reflect changes in the modelled concentration of ADP1.

as [ADP1] increases and the [ATP1] / [ADP1] ratio must therefore decrease as [P1] decreases.

In general terms, the relationship found in Figure (22) is consistent with data presented by Kaiser and Urbach [1977], which detail the interactions between [P1], [ATP1] and [ADP1]. In essence, they showed that when internal levels of P1 were reduced, the [ATP1] / [ADP1] ratio responded immediately by decreasing dramatically. They further found that the decline in [ATP1] / [ADP1] could be quickly reversed by increasing the external supplies of inorganic phosphate, equivalent to increasing [P2] in the model.

Further indirect evidence for the [ATP1] / [ADP1] ratio's dependence on [P1] and [PGA1] is shown in Figure (23).

From Figures (22) and (23), the overall relationship that emerges is a direct correspondence between [P1] and [ATP1] and an inverse proportionality between [P1] and [PGA1]. As explained in the model development section, the concentration of PGA1 is thermodynamically regulated. For the forward cyclic PGA1 reaction to proceed against a relatively large positive standard Gibbs' free energy change, the ratio in the following function

$$\Delta G^{\circ} + RT \ln \left(\frac{[BPGA] [ADP]}{[PGA] [ATP]} \right),$$

must be very large. This constraint couples [P1] levels directly to [PGA1] through its effect on the [ATP1] / [ADP1] ratio.

This explains the control path between chloroplasts and cytoplasm. [P1] is closely dependent on [P2] and by regulating the level of ATP1, [P1] can affect both the concentrations of intermediates and individual reaction rates as was shown in Figure (19).

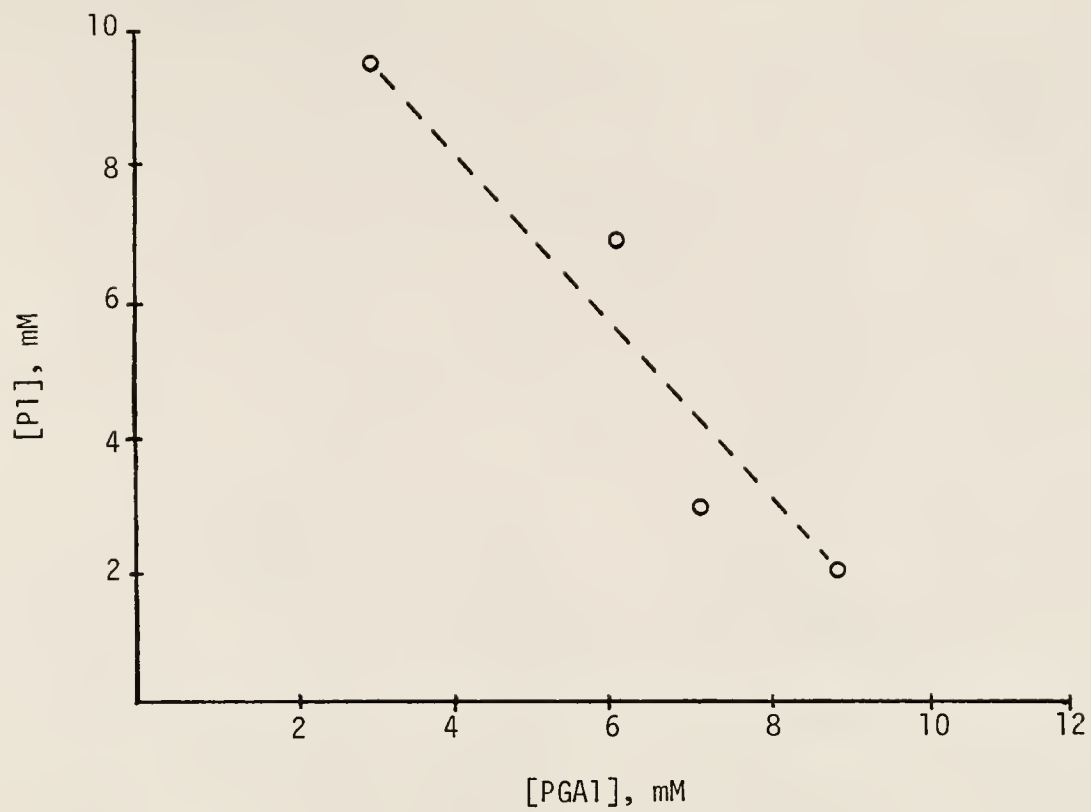


Figure 23. Measured chloroplastic PGA-inorganic phosphate response. Based on data from Heldt et al. [1977].

The following section considers how the cytoplasmic inorganic phosphate levels are regulated within the sucrose export system.

The Cytoplasmic Sucrose Balance, [SUCROSE]

Photosynthate that does not go to the starch tank flows to the cytoplasmic sucrose tank, from which it is exported to the rest of the plant. This balance is modelled by equation (43):

$$\frac{d[\text{SUCROSE}]}{dt} = \frac{\frac{1}{4} V_{dsmax} [\text{DHAP2}]}{[\text{DHAP2}] + K_{d2}(1 + [\text{SUCROSE}] / K_s)} - [\text{EXPORT}] , \quad (43)$$

where [EXPORT] equals the time rate of sucrose export from the cytoplasm. Under steady flow conditions, the sucrose tank is in steady state with imports from the chloroplasts just offsetting exports to the rest of the plant. In steady state, equation (18) can be simplified to the following:

$$[\text{EXPORT}] = \frac{\frac{1}{4} V_{dsmax} [\text{DHAP2}]}{[\text{DHAP2}] + K_{d2}(1 + [\text{SUCROSE}] / K_s)} . \quad (55)$$

Equation (55) has two variables and three constants on the right hand side. For purposes of analysis, assume that [DHAP2] is normalized by some average concentration such that, at an average concentration [DHAP2]_n equals 1. Further assume that V_{ex}max is approximately equal to the maximum rate of CO₂ fixation, V_{cr}max, which has been approximated in Table (6) to equal 300 μmol CO₂/mg chl/hr. K_{d2} and K_s have been assumed to equal 1.5 and 100 mM respectively, based partially on Hawker [1967]. The responsiveness of equation (55) can be tested with these values. Figure (24) illustrates the effect on [EXPORT] of varying

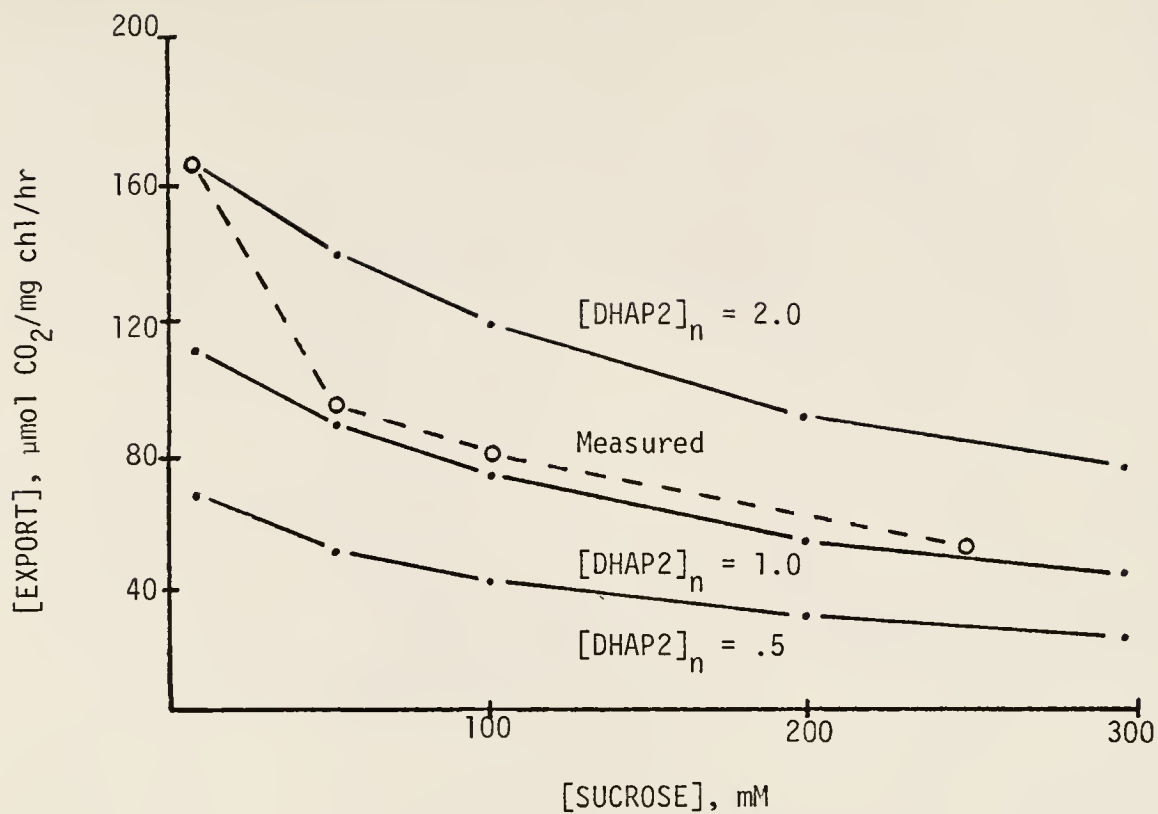


Figure 24. Measured and modelled sucrose concentration- $[\text{EXPORT}]$ response. Measured values (O) are from Hawker [1967]. Modelled values (·) are modelled for different normalized DHAP2 concentrations. $[\text{EXPORT}]$ is given in units of $\mu\text{mol CO}_2$ fixed and exported/mg chlorophyll/hour.

[SUCROSE] while $[DHAP2]_n$ is held constant, the dashed line is from data taken by Hawker [1967], who did pioneering work on feedback inhibition of sucrose formation.

Real World Scenarios

Much experimental work has been done on the relationships between photosynthesis, starch and sucrose. A question of particular interest is how partitioning between these photosynthetic end products and their respective concentrations might functionally affect photosynthetic rate. In researching this question, experimentalists have caused many different techniques to alter levels of starch and sucrose in a wide variety of plant materials and in settings ranging from laboratory to field conditions.

Some of the more common methods of changing leaf carbohydrate levels are manipulations of diurnal temperature regimes, ambient CO_2 concentrations and by manipulating the source-sink balance of carbohydrates. In each of these methods, enough work has been done to suggest certain general response patterns, although there are numerous exceptions which appear irreconcilable with the general trend. Direct manipulation of source-sink balance fits into the model more simply than temperature or CO_2 manipulation and will be considered first.

Direct Manipulation; Sucrose Feeding

In two specific experiments [Moore et al., 1974; Habeshaw, 1973], sucrose was directly applied to photosynthetic plant material. In both cases, increased levels of external sucrose depressed photosynthetic rate and increased carbohydrate concentrations in leaves. A large fraction of the increased carbohydrate was in the form of starch. A typical scenario can be applied to the model as follows.

The experimental plants are grown in some consistent environment, resulting in a particular balance between the sucrose exported from photosynthetic cells and the substrate and energy requirements of plant's growing points. By direct external application of sucrose, the concentration in the region around photosynthetic cells increases and apparent demand for sucrose decreases (perhaps by disrupting a diffusion gradient). This results in decreased export and cytoplasmic sucrose levels rise. High concentrations of sucrose cause [P2] to decrease, which can subsequently decrease [P1] enough to reduce photosynthesis. Low levels of P1 are also directly linked to increased levels of PGA1, which promote the accumulation of starch.

Direct Manipulation; Selective Shading

Another type of direct source-sink manipulation is the experimental work done by Thorne and Koller [1974], in which experimental plants grown in full light were completely shaded except for one leaf (the "source leaf"). Control plants remained unshaded. The "source leaf" on the experimental plant and a comparable leaf on a control plant were monitored for starch level, sucrose level, photosynthetic rate and inorganic phosphate level.

After eight days, the source leaves from the shaded plants had much lower levels of starch, higher levels of sucrose, higher levels of inorganic phosphate, and higher photosynthetic rates than did the source leaves from the unshaded control plants.

In terms of the model, this experiment poses an interesting problem. The sink demand was much higher for the source leaves on shaded plants than on the unshaded plants. According to the model, this should have decreased the cytoplasmic SUCROSE levels, increasing [P2] and [P1],

and decreasing [PGA1], all of which would facilitate photosynthesis and mobilization of starch.

All of these things happened, except [SUCROSE] increased steadily from 1% to 3% dry weight after nine days. This apparent inconsistency between the model and empirical results deserves close attention.

A central assumption in discussing the feedback biochemical controls of the sucrose system is that cytoplasmic inorganic phosphate levels are constant (in the short term). In this experiment, inorganic phosphate levels were six times higher in the shaded plant's source leaves than in the control plant after eight days of treatment. This means that both sucrose and inorganic phosphate levels increased significantly at the same time, which cannot be explained by the short term assumptions used in the model as derived. This apparently paradoxical situation could result simply from increased import of phosphate (perhaps being mined from the shaded leaves which would have high levels available).

With higher levels of P₂, larger pools of phosphorylated sucrose antecedents can be maintained without affecting photosynthetic rates via control of P₁ concentration. From Figure (24), it is clear that export rates are increased by high concentrations of DHAP₂, which represents the pool of phosphorylated sucrose antecedents.

For the shaded plants in the Koller and Thomas experiment sink demand was high. Even so, the source leaf maintained relatively higher levels of sucrose than the control. This gradient promoted relatively rapid export, which was evident in the higher CO₂ exchange rates found for the shaded plant's source leaves. At normal phosphate levels, the high sucrose concentration maintained in these leaves would reduce available inorganic phosphate, causing starch formation and reducing

photosynthesis. Importing phosphate would alter the balance of sucrose to phosphorylated antecedents that could be tolerated without affecting P2 and P1 concentrations. Higher antecedent levels might drive sucrose formation or appropriate enzyme levels might be increased, circumventing the inhibition caused by the high sucrose levels. The model as derived can mimic this experimental situation on any given day, but constants related to enzyme levels and phosphate levels which are valid in the short term could not follow the experiment through eight days.

Temperature Manipulation

As reviewed by Patterson [1980], temperature manipulation experiments frequently involve growing plants under one set of day/night temperatures and then switching the acclimated plants to a different pair of day/night temperatures. In general, changing the temperature environment does affect photosynthetic rate, starch level and sucrose level. One typical scenario might be as follows. Plants are grown in a warm environment, for instance, 25/15° C day/night temperature. Carbohydrate levels and CER are monitored. The temperature environment is then changed to a cold night regime, 25/5° C, and as before, appropriate measurements are taken. Expected results on the morning following the first cold night might be as follows.

Photosynthetic rate would be suppressed [Rook, 1969], starch levels would be higher than following a warm night [Hilliard and West, 1970], and soluble carbohydrates, including sucrose concentrations would be relatively higher [Barlow and Boersma, 1976].

This proposed scenario is consistent with model behavior; increased levels of sucrose should increase the concentrations of phosphorylated sugars, decreasing [P2] which leads to increased starch accumulation and decreased photosynthetic rate, as already described.

CO₂ Concentration Manipulation

As reviewed by Guinn and Manney [1980], several CO₂ enrichment experiments are described in which plants are grown in a particular CO₂ concentration and then exposed to a different CO₂ concentration.

An experiment by Mauney et al. [1980] is representative. Cotton plants were grown in an atmosphere with 330 vpm CO₂, then were transferred to an atmosphere of 630 vpm CO₂. During the first 2 hours after transfer, the CER increased to a level 45% higher than the rate at low CO₂ levels. The rate then began a slow decline "as starch accumulated," until net photosynthesis was only 15% above the rate at low CO₂ levels. The plants were then switched back to 330 vpm CO₂ and photosynthetic rate dropped below the initial rates in low CO₂ conditions. After three days, the plants regained the initial rates of photosynthesis.

In terms of a modelled scenario, increased concentration of CO₂ causes proportionately more RuBP reduction of CO₂, and proportionately less RuBP reduction of O₂. Thus, more CO₂ can be fixed per molecule of RuBP produced by the Calvin cycle and consequently, less ATP energy units are required per unit of CO₂ fixed. The result is to increase levels of important chloroplastic intermediates which facilitate increased export to the cytoplasmic sucrose tank. As long as sink demand is high, sucrose export rates are high and, as in the example above, the photosynthetic rate is high. However, as the sink requirements are met, sucrose exports decline and cytoplasmic sucrose levels increase until a new source-sink balance is established; in the experiment above, the new balance had a steady state photosynthetic rate 15% higher than the initial rate.

In the experiment's subsequent decrease in CO₂ level, the plants were returned to initial external conditions, but internal starch level

was measurably higher. As a result the source-sink balance was altered. In this model starch accumulation results from increased sucrose levels which affect the chloroplastic levels of P1 and PGA1 and increased sucrose levels result from decreased export, which is a function of low sink demand. In terms of the model, the plant's internal sink demand was low and starch levels were high. Photosynthetic rates were lower than had been the case initially. Until the plant was able to assimilate the excess supplies of sucrose and starch, cytoplasmic sucrose levels remained high, and [P1] remained low.

Finally, after three days, the starch levels dropped back to initial levels, indicating that the excess carbohydrate had worked its way through the system and the original source-sink balance was re-established.

The preceding scenarios have shown how the proposed biochemical model could simulate alterations in carbohydrate production, utilization and partitioning caused by diverse changes in external conditions. This analysis emphasizes that starch and sucrose have integral roles in the biochemistry of photosynthesis and are definitely not uninvolved products. The proposed model offers a direct mechanistic explanation for end product (starch and sucrose) inhibition of photosynthesis [Guinn and Mauney, 1980]. Furthermore, this analysis indirectly offers some insight into types of possible adaptation to changed environmental conditions. This idea will be explored further in the results from the experimental field work.

EXPERIMENTAL METHOD

Procedure

Field experiments designed to investigate short term photosynthetic response and long term photosynthetic adaptation were conducted from February through May of 1980. Data were taken which can be used for further qualitative evaluation of the model developed in the preceding chapters.

Soybeans (Glycine max L., cv. Bragg) were planted on February 29, 1980 in four environmental control chambers located outdoors so that they were irradiated by natural solar radiation. Plants were spaced equidistantly 0.1 m from each other and 0.05 m from the chamber walls. This arrangement produced a grid of 5 rows (N-S) and 20 columns (E-W) which were designated by number (1-5) and letter (A-T). Plants along the canopy edge were not used for any direct measurements or sampling. Each unit was exposed equally to ambient irradiance, adequate nutrients and similar soil water potentials throughout the experiment. Dry bulb temperatures were controlled at 25° C both day and night. Chamber CO₂ concentrations were maintained at 320 vpm in chamber 3 (Low-Low) and at 640 vpm in chamber 2 (High-High). CO₂ levels in chambers 1 (Low-High) and 4 (High-Low) were held at 320 vpm and 640 vpm, respectively, until 7:00 a.m. on April 22, when their CO₂ concentrations were reversed for the remainder of the experiment. The experiment ended on May 10, 1980, when the plants in all chambers were harvested.

From March 18 through April 9 vertical distribution of leaf area was measured on four plants in specific grid positions within each chamber. Measurements were made on the leaf area of the central leaf in each trifoliate, internode lengths and overall height. These values were used to calculate leaf expansion rates and to estimate leaf area index, total leaf area per unit of projected ground area (LAI).

Diurnal samples were taken on six different days: April 11, April 16, April 21, April 22, April 29 and May 6. These days all had cloudless mornings while afternoons had intermittent cloud coverage. Three trifoliate leaves from upper sunlit leaves and three from lower shaded leaves were sampled. These samples were taken three times during each diurnal sampling period at 8 a.m., 2 p.m. and 5 p.m. In addition, at 11 a.m., two whole plant samples were taken from each chamber. All samples were placed in labelled brown paper sacks and kept on ice until all four chambers were sampled. Leaf area was measured with a Lambda Alpha digital planimeter (model LI-3000). Samples were dried for 24 hours at 90° C, placed in plastic bags and refrigerated until they could be weighed to an accuracy of 0.000g on a Mettler analytical balance.

The 11 a.m. whole plant samples were subdivided into groups based on height distribution. The lower three trifoliate leaves from both plants were lumped together as subsample (A), the fourth and fifth trifoliate leaves were grouped as (B), the sixth and seventh (C), and so forth. Before drying, these subsamples were measured for chlorophyll content, using the procedure of Arnon [1949]. After drying, all samples were weighed and specific leaf weights were calculated. Dry, weighed leaves were ground in a Wiley Mill to a fine powder to pass a one mm screen in preparation for laboratory analysis. The subsamples were measured for nitrogen

with micro Kjeldahl technique, and for inorganic phosphate with a colorimetric technique [Tavssky and Shorr, 1953]. Selected samples were analyzed for total available carbohydrates using the enzymatic procedure described by Smith [1969] for extraction and a colorimetric test for measurement [Nelson, 1944; Somogyi, 1952].

Physical Characteristics

Experiments were conducted in four plexiglas chambers similar to the Soil Plant Air Research (SPAR) units developed at Clemson [Phene et al., 1978]. The units measured 2.0 m by 0.5 m in cross section by 1.5 m tall. The plexiglas panels were joined with aluminum angle braces and all joints were well sealed with silicon caulk. The chambers could be entered through the south panel (2.0 m by 1.5 m), which was secured with bolts and sealed with a neoprene gasket, or through two small panels (0.15 m by 0.15 m) on the north side, which were secured by studs with wingnuts and sealed with neoprene gaskets. Air circulation was provided at the rate of 4 air changes per minute by a continuously operating squirrel cage fan housed in a duct on the chamber's north wall. Inlet and exit baffles at chamber top and bottom insured good mixing. The insulated duct work also contained a heat exchanger (cooling coil), resistance heater, sample gas exit port, condensate exit port and water injection system. The duct was also equipped with a piston operated venting port (0.3 m by 0.2 m), which utilized pressure gradients around the circulation fan to flush the chamber with ambient air. A side view schematic of a chamber is given in Figure (25).

Each chamber was secured to a soil lysimeter 2.0 m by 0.5 m in cross section by 1.0 m deep, constructed of steel and equipped with a port for the pressurized injection of irrigation water and nutrient

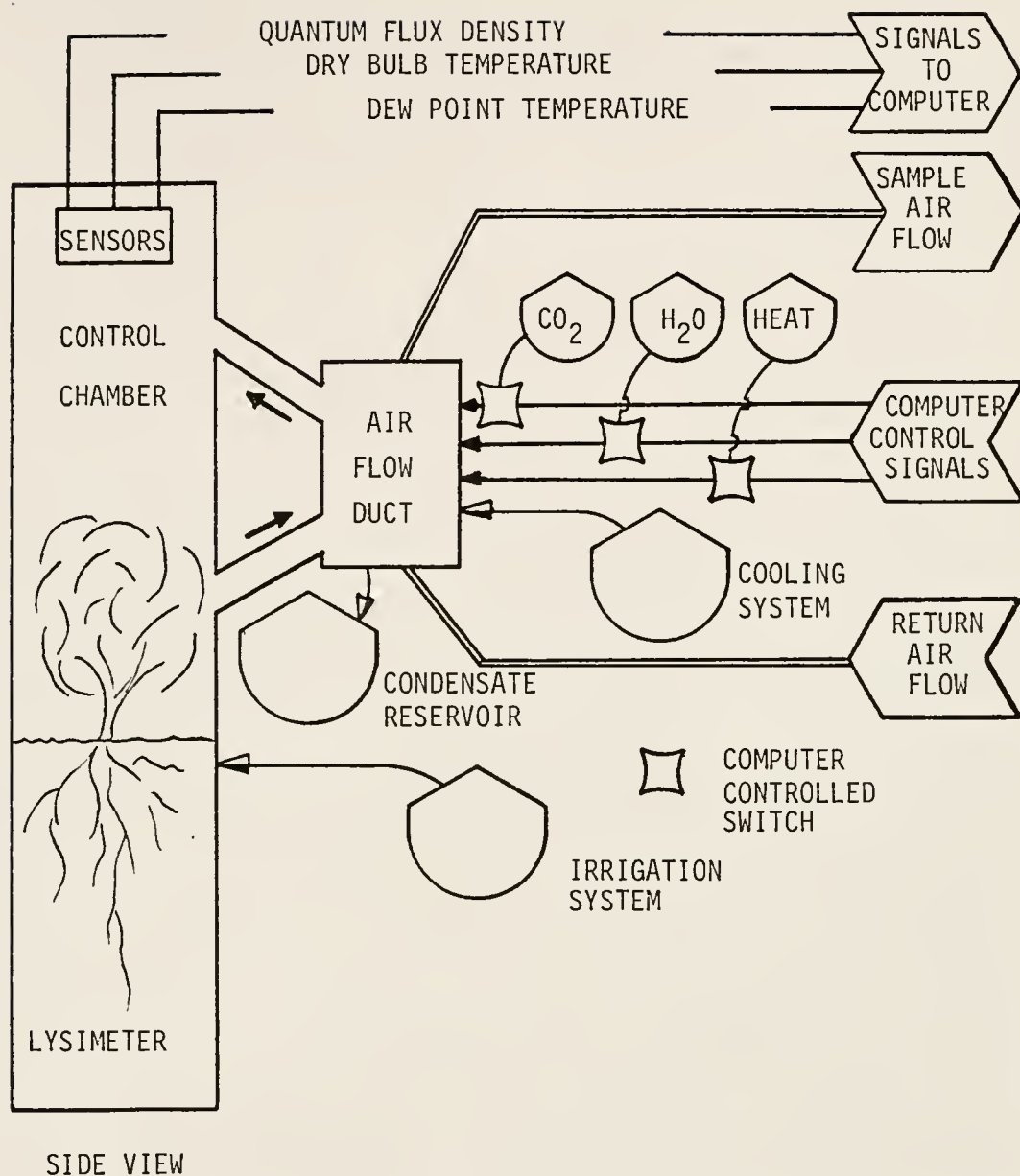


Figure 25. Control chamber schematic. Chamber environment was controlled by a computer which was programmed to respond to four basic sensor signals: quantum flux density, dry bulb temperature, dew point temperature and a measurement of CO_2 concentration. Based on these signals the computer regulated the input of CO_2 , H_2O and heat to the chamber to maintain desired conditions. The sample air flow line circulated air from the control chamber to a gas analyzer which sent a millivolt signal to the computer. More detail is provided in Figure (26).

solution, ports for tensiometers at 6 depths spaced 0.15 m apart, as well as a drain valve from which accumulated water could be removed through porous ceramic candles by vacuum pump. The lysimeters were placed in pits 2.0 m by 2.0 m in cross section by 1.0 m deep, with an access space on the north side of the system.

Controlled Environmental Factors

The main parameters controlled in this study were dry bulb temperature and CO₂ concentration inside the chambers. Both were controlled by digital computer, using feedback algorithms in conjunction with appropriate sensors. Each chamber's dry bulb temperature was measured with a thermocouple located near the exit air baffle at chamber bottom. Every 10 seconds temperatures were measured and control decisions made for each chamber. The control decision was whether the resistance heater should be on or off since the cooling coils operated continuously. The decision algorithm was based on both current temperature and the rate of temperature change over the preceding 10 second interval.

CO₂ controls were somewhat more complex. They operated in two automatic modes, one for daytime photosynthesis measurements and the other for night time respiration measurements. In addition, the automatic controls could be overridden by manual controls, which allowed special manipulations of chamber CO₂ levels. CO₂ concentration was sensed with a Beckman Model 865 infrared gas analyzer housed 20.0 m from the chambers in an instrumentation trailer. Each chamber was connected to the trailer by a continuous flow, closed gas sampling circuit. Solenoids sequentially diverted sample gas from each of the four chambers through the analyzer. Each circuit was sampled for 75 seconds to allow for flushing and instrument stabilization. A complete sampling cycle

of the four chambers took 5 minutes. A schematic representation of the control system is given in Figure (26).

Carbon dioxide exchange rate (CER) for each 5-minute cycling interval was calculated using conservation of mass as in the following equation:

$$\text{CER} = (\Sigma \text{MCO}_2\text{inj} + \text{MCO}_2\text{i} - \text{MCO}_2\text{e}) / (.083)(\text{AREA})(5 \text{ min}) \quad (56)$$

where $\Sigma \text{MCO}_2\text{inj}$ = the sum of CO_2 injected during the 5-minute cycle,
kg/5 min,

MCO_2i = the initial mass of chamber CO_2 for current 5-minute cycle, kg,

MCO_2e = the mass of chamber CO_2 at the end of the current 5-minute cycle, kg,

0.083 = the conversion of the 5-minute cycle time to units of hours, hr/5 min,

AREA = chamber ground surface area, m^2 , and

CER = CO_2 exchange rate, kg/hr/m^2 .

An analysis of probable error is given in Appendix 5.

During the intervals between measurements, CO_2 concentration was maintained at desired levels using a control algorithm based on the level of photosynthetically active radiation (PAR). Every 10 seconds PAR was measured and used to predict CER for each chamber via an empirically derived functional equation. The equation can be written as follows:

$$\text{PCER} = (\text{PNMAX} * \text{PAR} / (\text{KPAR} + \text{PAR})) - \text{RC} \quad (57)$$

where PCER = predicted CER, $\text{kg/m}^2/\text{hr}$,

PNMAX = maximum possible CER, $\text{kg/m}^2/\text{hr}$,

PAR = quantum flux density of photosynthetically active radiation, $\mu\text{E/m}^2/\text{hr}$,

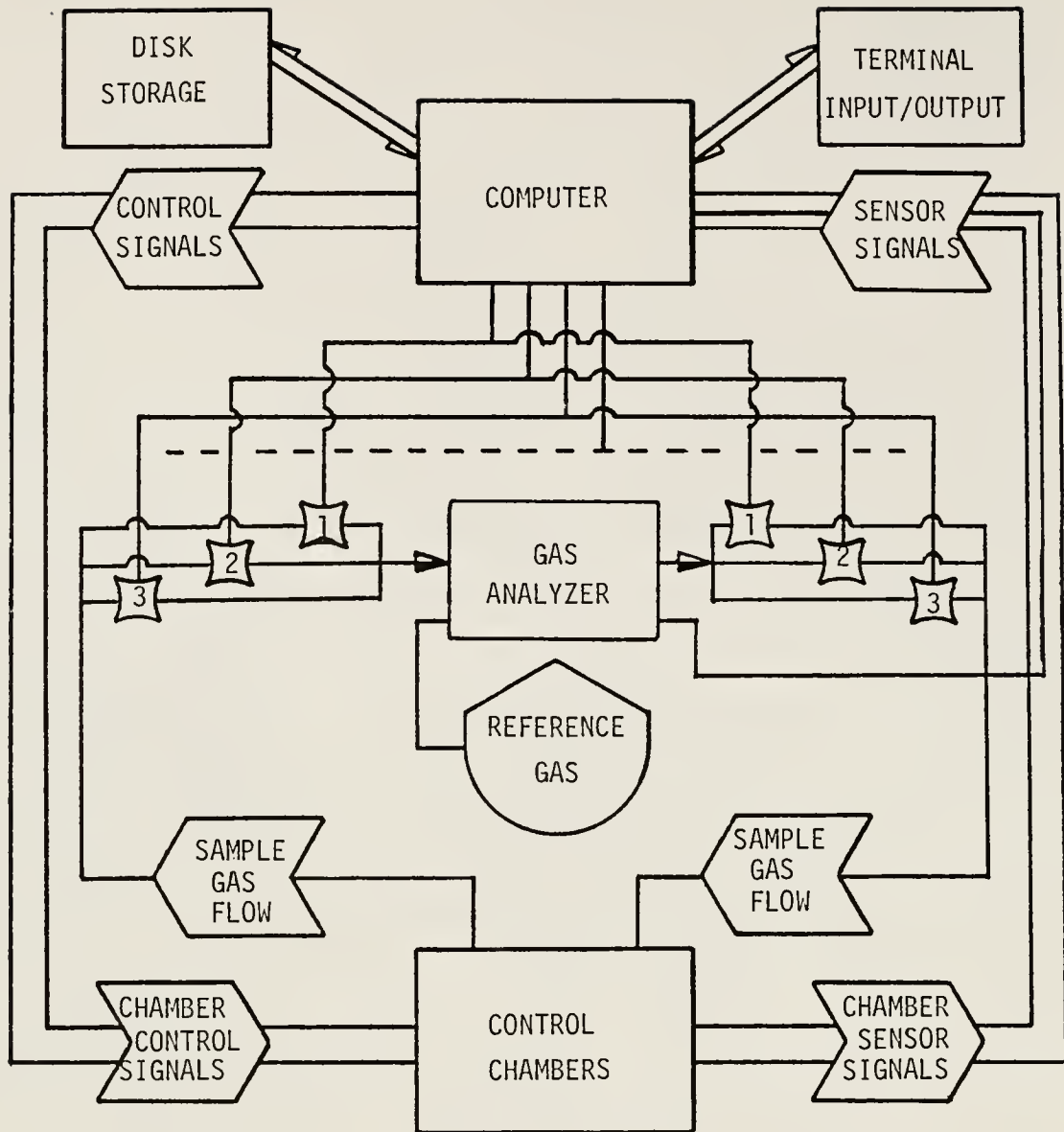


Figure 26. Control system schematic. Sensor signals were used in a digital feedback circuit to control various inputs to the control chambers. The computer sampled $[CO_2]$ in each chamber by sequentially opening sets of solenoids ($\mathbf{\text{H}}$).² Data were stored on disks. The system was completed with a terminal which provided access to data and to the control program in the computer.

KPAR = PAR for half maximal CER, $\mu\text{E}/\text{m}^2/\text{hr}$, and

RC = Respiration related correction coefficient, $\text{kg}/\text{m}^2/\text{hr}$.

Based on the predicted CO_2 exchange rate, and a correction factor to account for drifts detected by the 5-minute measurements, the 10-second CO_2 injection requirement, $(\text{MCO}_2)_{\text{inj}}$, was calculated. In equation form,

$$\text{MCO}_2_{\text{inj}} = \text{PCER} * \text{AREA}/360. + ([\text{CO}_2]_{\text{d}} - [\text{CO}_2]_{\text{m}}) * \text{VOLUME}/10 \text{ sec} \quad (58)$$

where PCER = predicted CER, $\text{kg}/\text{m}^2/\text{hr}$,

AREA = chamber ground surface area, m^2 ,

360. = conversion from an hourly to a 10 second basis, 10 sec/hr,

$[\text{CO}_2]_{\text{d}}$ = desired CO_2 concentration, kg/m^3 ,

$[\text{CO}_2]_{\text{m}}$ = measured CO_2 concentration, kg/m^3

VOLUME = chamber volume, m^3 , and

$\text{MCO}_2_{\text{inj}}$ = mass of CO_2 injection for current 10-second interval, $\text{kg}/10 \text{ sec}$.

Dark respiration was measured at night over 15 minute periods each hour. The chambers were each equipped with piston-operated venting ports which were activated by computer control of solenoids in pressure supply lines to the pistons. When the vents were opened, pressure gradients created by the circulation fan flushed the chambers with ambient air. Computer controls sequentially opened each chamber vent for 15 minutes, closed the vent, and then CO_2 concentrations were measured continuously for 15 minutes. For this process, CO_2 concentration was recorded on strip charts that showed the increase in chamber CO_2 concentration which resulted from dark respiration. One hour was required to complete each cycle of respiration measurements for all four chambers. As with photosynthetic rate, calculation of dark respiration required a simple mass balance. The change in chamber CO_2 level during the 15-minute cycle was directly equated to dark respiration (RD) by:

$$RD = ([CO_2]_e - [CO_2]_0) * VOLUME * 4.0 / AREA \quad (59)$$

where $[CO_2]_e$ = chamber CO_2 concentration at the end of the 15-minute interval, $kg/m^3/15 \text{ min}$,

VOLUME = chamber volume, m^3 ,

4.0 = the conversion factor from a 15-minute to an hourly basis,

AREA = chamber surface area, m^2 ,

RD = dark respiration rate, $kg/m^2/hr$, and

$[CO_2]_0$ = chamber CO_2 concentration at the beginning of the 15 minute interval, $kg/m^3/15 \text{ min}$.

Since the chambers were not perfectly air tight, leakage exchanges were expected. The following procedure was used to estimate leakage exchange effects. After the respiration rate was determined with the night time technique described above, CO_2 was injected until chamber concentration was at 660 vpm. Then CO_2 level was continuously monitored for 15 minutes and recorded by strip chart. The initial slopes of the tracings were always negative, indicating that leakage was removing CO_2 from each chamber more rapidly than dark respiration could supply it. The rate of total leakage loss (DL) was equal to the absolute sum of the drawdown rate at 660 vpm (the slope of the recorded CO_2 concentration) and the respiration rate.

$$DL = [\dot{CO}_2]_m^{660} * VOLUME / AREA + RD \quad (60)$$

where $[\dot{CO}_2]_m^{660}$ = chamber CO_2 drawdown rate measured at 660 vpm, $kg/m^3/hr$,

VOLUME = chamber volume, m^3 ,

AREA = chamber surface area, m^2 ,

RD = dark respiration rate, kg/hr/m**2,

DL = diffusive leakage rate, kg/hr/m**2.

The leakage rate was considered to be a function of the gradient between chamber and ambient levels of CO₂. Since this driving force was relatively stable and constant for each of the chambers, the differences in leakage rate were treated as a function of differing resistances among the chambers. Based on this assumption, diffusional leakage can be expressed as follows:

$$DL = ([CO_2]_d - [CO_2]_a)/CR \quad (61)$$

where $[CO_2]_d$ = desired chamber CO₂ concentration, kg/m**3,

$[CO_2]_a$ = ambient CO₂ concentration, kg/m**3,

CR = chamber resistance to diffusion, hr/m,

DL = diffusional leakage rate, kg/hr/m**2.

The resistance value for each chamber was obtained by combining equation (60) and (61) to yield

$$CR = ([CO_2]_d - [CO_2]_a)/([\dot{CO}_2]_m^{660} * VOLUME/AREA + RD) \quad (62)$$

In order to approximate daytime chamber leakage, the resistance value for each chamber was assumed to be constant. Then, using typical daytime ambient CO₂ concentrations, 330 vpm ($6.5 * 10^{-4}$ kg/m**3) for $[CO_2]_a$ and the desired chamber values, $[CO_2]_d$, the daytime leakage rate for each chamber was calculated. The chambers were leak tested every 10 days to reconfirm their resistance values. (See Appendix 5 for error analysis.)

Soil Respiration

The soil zone was separated from the upper canopy by a plexiglas barrier supported about 25 mm above the lysimeter soil. Plants grew

through appropriately spaced holes which were sealed with foam and hot wax when the plants were two weeks old. Air was drawn from a height of 6 m and was pumped through a 30 gallon buffering tank to damp out local fluctuations in ambient CO_2 concentrations. From the buffering tank, air was pumped to each chamber through an inlet port at the east end of each lysimeter, through the soil/plexiglas air space and out a port in the west end of each lysimeter. CO_2 flux from the soil was measured, using an infrared gas analyzer (Beckman Model 865) which pulled reference gas from the buffering tank and sample gas from each chamber's exit port. Chambers were monitored automatically with a computer controlled multiplexer which delivered sample gas from each chamber in sequence.

Water

Transpiration rates were measured manually by collecting condensate from each chamber's cooling coils in a graduated cylinder for a specified time interval (5 minutes). This was usually done three times each day. When the overflow was not being caught in a cylinder, it flowed into a large (18 liter) storage container. This system provided an approximate measure of total daily transpiration. To maintain a soil water balance, irrigation water was applied to replace the transpiration losses. Vertical distribution of the soil water was measured with tensiometers. Accumulated water at the bottom of the lysimeter was periodically removed through the drain valve. Dew point measurements were taken using a dew point hygrometer which measured gas in the same sampling circuit used for CO_2 analysis. Tensiometer and dew point measurements were taken simultaneously with the graduated cylinder transpiration values.

Photosynthetically Active Radiation (PAR)

Quanta flux density of photosynthetically active radiation (PAR) was measured above canopy with a Lambda Alpha quantum sensor (Model LI-1905). Values were taken every 10 seconds and used for control of CO₂ injection. At the end of a 5 minute CO₂ sampling cycle, values were averaged and stored as data on disks.

Problems

Diverse problems were encountered during the 10 weeks of field work. Some of the difficulties arose from simple and unavoidable mechanical failure, while others were technique or management errors. Each of the problems affected results in a unique manner and to a different extent. Some had a direct and recognizable effect, while others may have caused biases over time which were not distinguishable.

In the control system, air conditioners were operated continuously in every chamber, with the computer turning reheaters on or off based on thermocouple readings from each chamber. On occasions when electrical power was interrupted, the control program would crash, which left the air conditioner running without any heater control. This caused chamber temperatures to drop. In the worst case, temperatures fell to approximately 13° C, at that point the air conditioner thermostats began to control. Another difficulty with temperature control occurred when computer storage disks were filled, which caused the computer to crash without a power failure. This allowed heater control bits within the computer to remain set. If a heater was on under these circumstances it remained on until the program was brought up or power was interrupted. Most of the time disks were changed before they were filled, however on a few occasions the system did crash. At its worst, this problem

allowed the temperatures in two chambers to climb to approximately 45° C for a period of several hours.

At the mechanical level, air conditioner coils occasionally froze, which allowed daytime temperatures inside the chambers to climb. Even without supplemental heating, chamber temperatures at midday rose above 40° C whenever an air conditioner was down. This problem occurred infrequently.

A more troublesome mechanical problem occurred when diaphragms ruptured in the sample line pumps. This allowed pumping to continue, but mixed ambient with chamber air. Since CO₂ levels were controlled based on measurements of air in the sample lines, this caused the high CO₂ chamber air to be diluted and controls injected excessive CO₂ into those chambers. Usually, one diaphragm had to be replaced each week.

Another problem with CO₂ controls resulted from the leaks that developed in the chambers themselves which allowed outside air to dilute CO₂ levels in the high CO₂ chambers. Leaks occurred in two ways: plexiglas joints failed, and the automated mechanical port used for night time respiration measurements failed to seat and seal properly.

The most damaging problem encountered during the experiment occurred while searching for chamber leaks. The technique used was to inject freon and use a freon detector to search for leaks around joints and seals on the outside of each chamber. Unfortunately, the by-products of burning freon are noxious. This caused no problem when burning outside the chambers, but in one chamber the heater was on and the injected gas severely burned the canopy's new growth. This occurred on the evening of April 16 in chamber 2 (H-H) and affected the transpiration and photosynthetic rates for several days. The plant canopy seemed to have recovered by April 21.

A second problem involving direct damage to plants occurred from late April through early May. In chambers 1 (L-H) and 2 (H-H) there was an infestation of spider mites. As before, one of the first indications was a change in transpiration and photosynthetic rates. The mites were eliminated by May 2 and some recovery was evident by May 6.

It is not clear how much effect these problems had on the overall plant response in the chambers involved.

Finally, the experimental procedure itself had some shortcomings. The chambers were planted thickly so the canopy would close quickly. This worked well. However, it caused the plants to grow tall and spindly. When whole plant samples were taken from the chambers, other plants were damaged. They were not broken, but tended to fall over somewhat, causing the canopy architecture to be changed. Secondly, shading was not provided around the edge of the chambers as the canopy grew which means that portions of the canopy leaf area exposed to direct PAR changed during the day. This is probably unimportant on a relative basis when comparing results between chambers, but undoubtedly affected absolute values.

One of the values most affected was leaf area index. Values obtained were at least twice as large as in normal field canopies. This is not too surprising when the total exposed canopy area is considered. When the canopies were half a meter tall the fully sunlit area was approximately $2\frac{1}{2}\text{m}^2$ with an additional $1\frac{1}{2}\text{m}^2$ of indirectly exposed edges. In a field canopy the fully sunlit area is 1m^2 . In future work edge effects must be carefully considered and avoided if possible.

EXPERIMENTAL RESULTS

Controls

Controls worked well throughout the experimental procedure; desired temperature and CO₂ conditions seldom varied by more than 5% from control levels. Temperature was maintained within $\pm 1^\circ$ of the desired value of 25° C, as shown in Figure (27). CO₂ levels in the high concentration chambers were usually within ± 20 vpm of the desired level of 640 vpm, while the low concentration chambers were usually within ± 10 vpm of the 320 vpm control level. Examples of $[CO_2]_m$ as a function of time for both high and low concentration chambers are given in Figures (28) and (29). CO₂ controls worked least well early in the morning (until 7:30 a.m. or thereabouts) and under rapidly changing light levels.

Carbon Balance

On April 22, the CO₂ concentrations in chambers 1 (L-H) and 4 (H-L) were switched from 320 vpm to 640 vpm and from 640 vpm to 320 vpm, respectively. In order to compare canopy response before and after the transition, the switch was made during a period of clear weather. Time courses for photosynthetically active radiation (PAR) $[CO_2]_1$, $[CO_2]_4$ and the carbon dioxide exchange rates (CER) for each chamber on April 21 are given in Figure (28). A parallel set of measurements for the period following the switch on April 22 is given in Figure (29). Comparing the measured light levels in the two figures confirms that the two days were virtually identical.

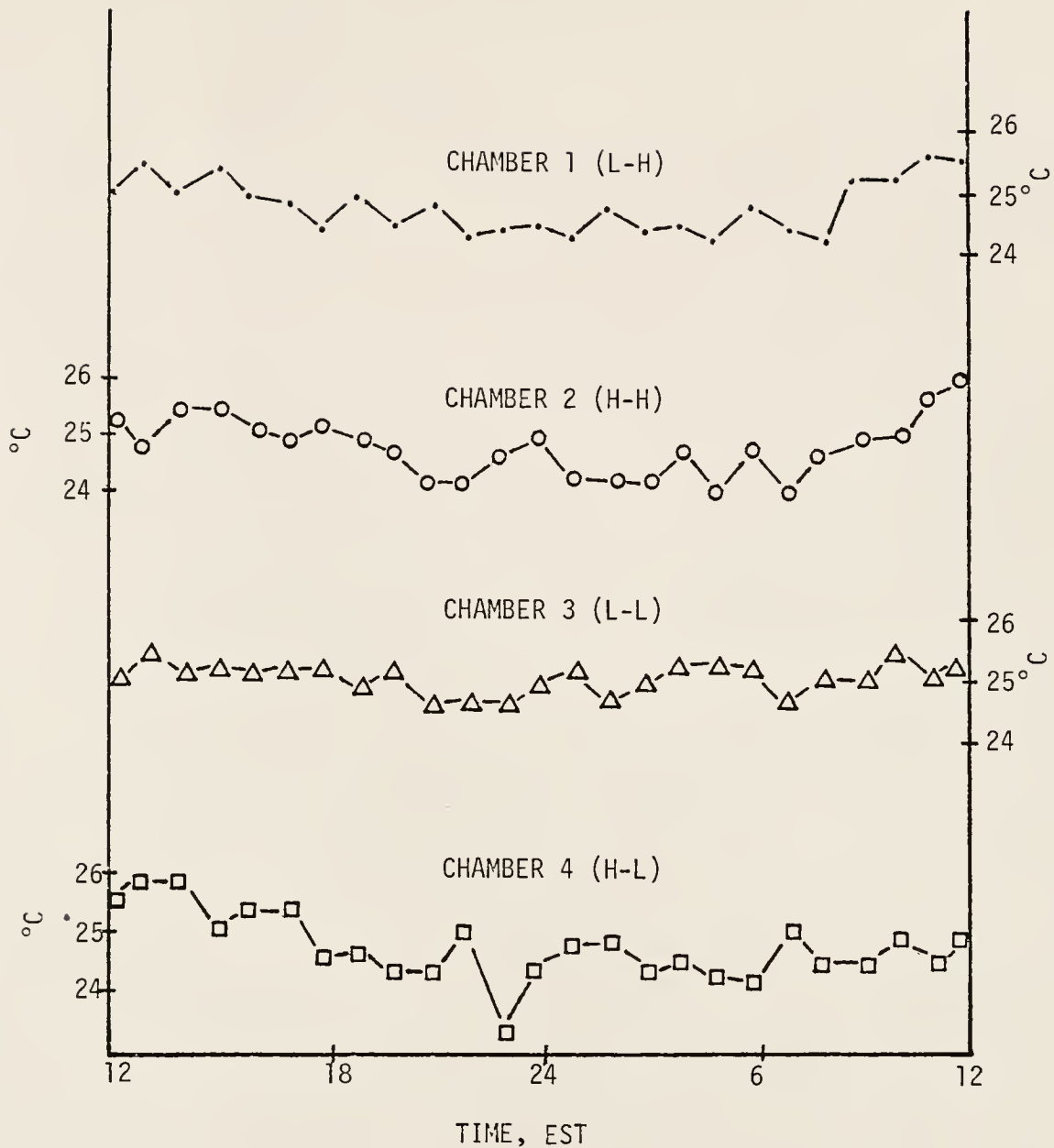


Figure 27. Diurnal temperature control. Hourly measured chamber air temperatures from noon on April 21 until noon on April 22. The desired control temperature was 25° C for all chambers.

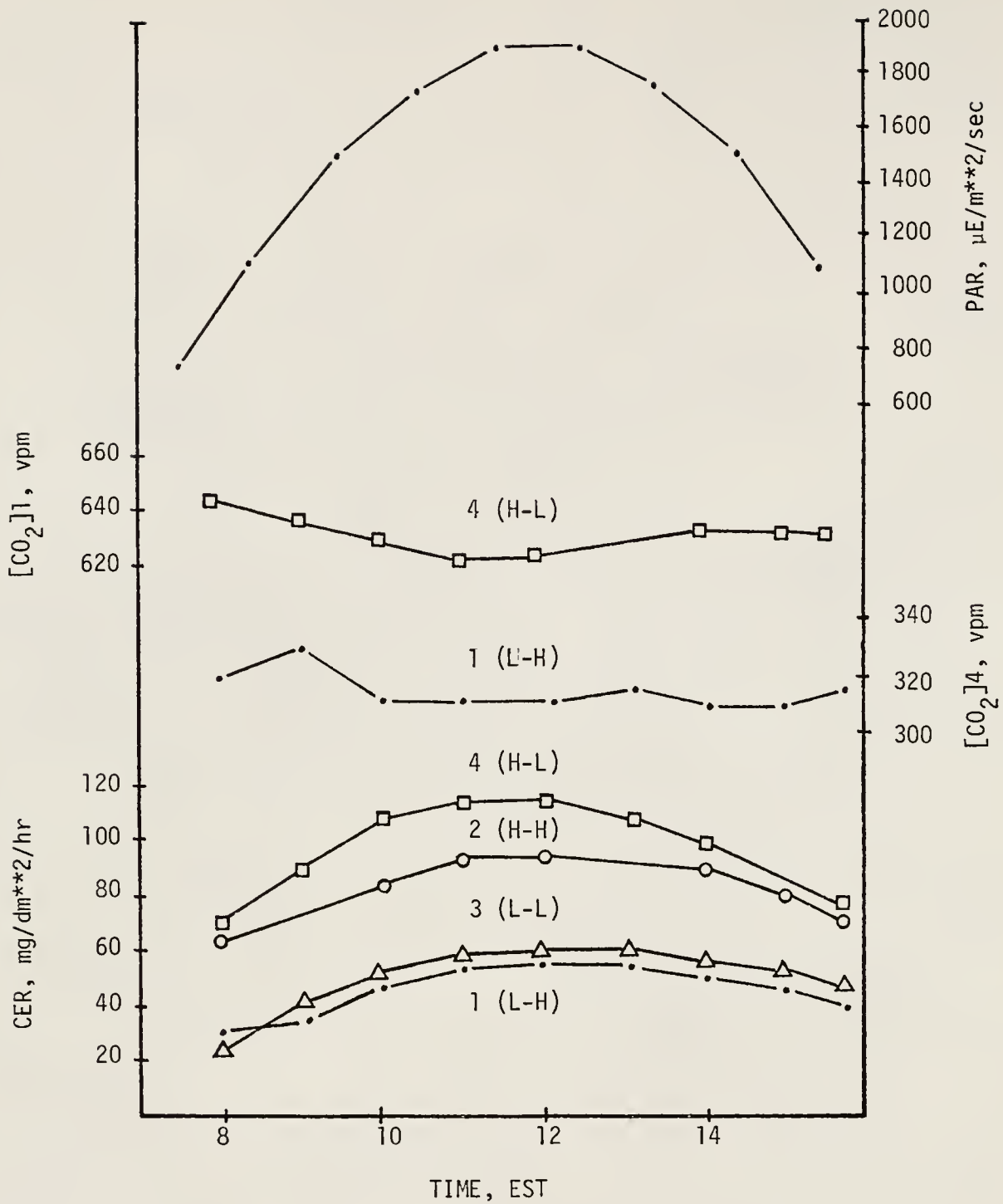


Figure 28. Time courses of PAR, $[CO_2]$ and CER on April 21. This is the day preceding the transition in CO_2 levels. CER is given in units of $mg\ CO_2$ fixed/ dm^2 land area/hr.

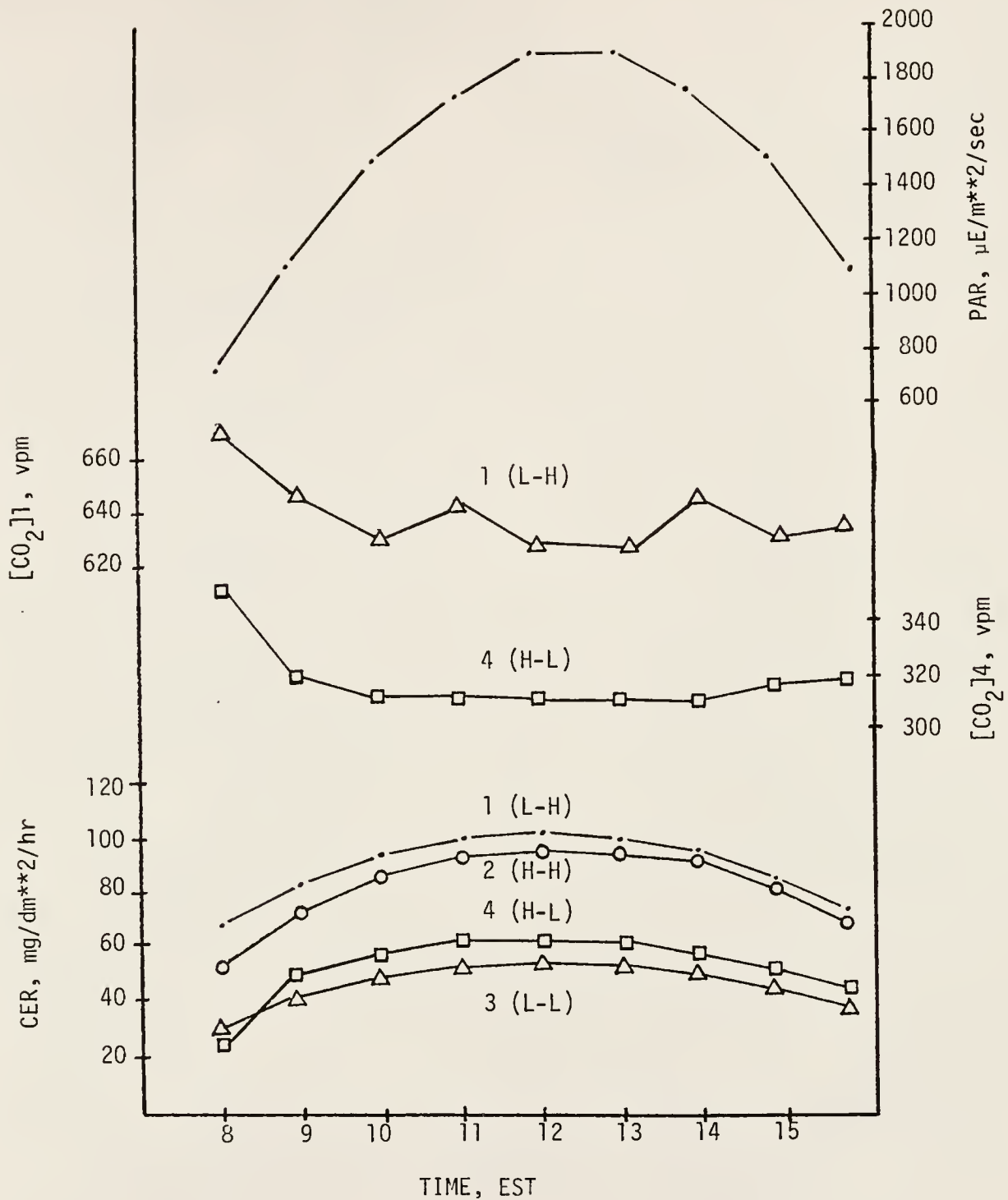


Figure 29. Time courses of PAR, $[CO_2]$ and CER on April 22. This is the day following the transition in $^{14}CO_2$ levels. CER units are given in units of $mg CO_2$ fixed/ dm^2 land area/hr.

The graphs of each chamber's CER on the two days demonstrate the strong correspondence between CO_2 concentration and net photosynthesis. Before the switch, chambers 2 (H-H) and 4 (H-L) maintained a net photosynthetic rate approximately twice as great as chambers 1 (L-H) and 3 (L-L). After the switch, chambers 1 (L-H) and 2 (H-H) had the high CO_2 levels and the high net photosynthetic rates, while the chamber 4 (H-L) rate dropped down to the level of chamber 3 (L-L). Response to the change in CO_2 concentration was immediate. Another feature seen in these graphs is that CER seems to peak somewhat before light level. This effect will be considered in a later section.

Figures (28) and (29) also demonstrate the close relationship between PAR and CER. Figures (30) and (31) provide a closer examination of this relationship over a 12 day period in a series of photosynthetic light response curves given for each chamber. Curves for April 16, 21, 22 and 28 are given for chambers 1 (L-H) and 2 (H-H) in Figure (30) and for chambers 3 (L-L) and 4 (H-L) in Figure (31).

Looking first at chamber 1 (L-H) in Figure (30), the separation between high $[\text{CO}_2]$ response on April 22 and 28 and low $[\text{CO}_2]$ response on April 16 and 21 is striking. A less obvious feature is the increased photosynthetic response to the same light level over time, indicating the continued growth of the canopies, both before and after the switch. Chamber 2 (H-H) does not show dramatic separation like chamber 1 (L-H) since the CO_2 concentration was constant at 640 vpm. However, the increased photosynthetic response over time is clear, with the response on April 16 being lowest and on April 28 being the highest. Before the switch chamber 2 (H-H) was roughly twice as responsive as chamber 1 (L-H), but after the transition the chambers' responses became nearly identical by April 28.

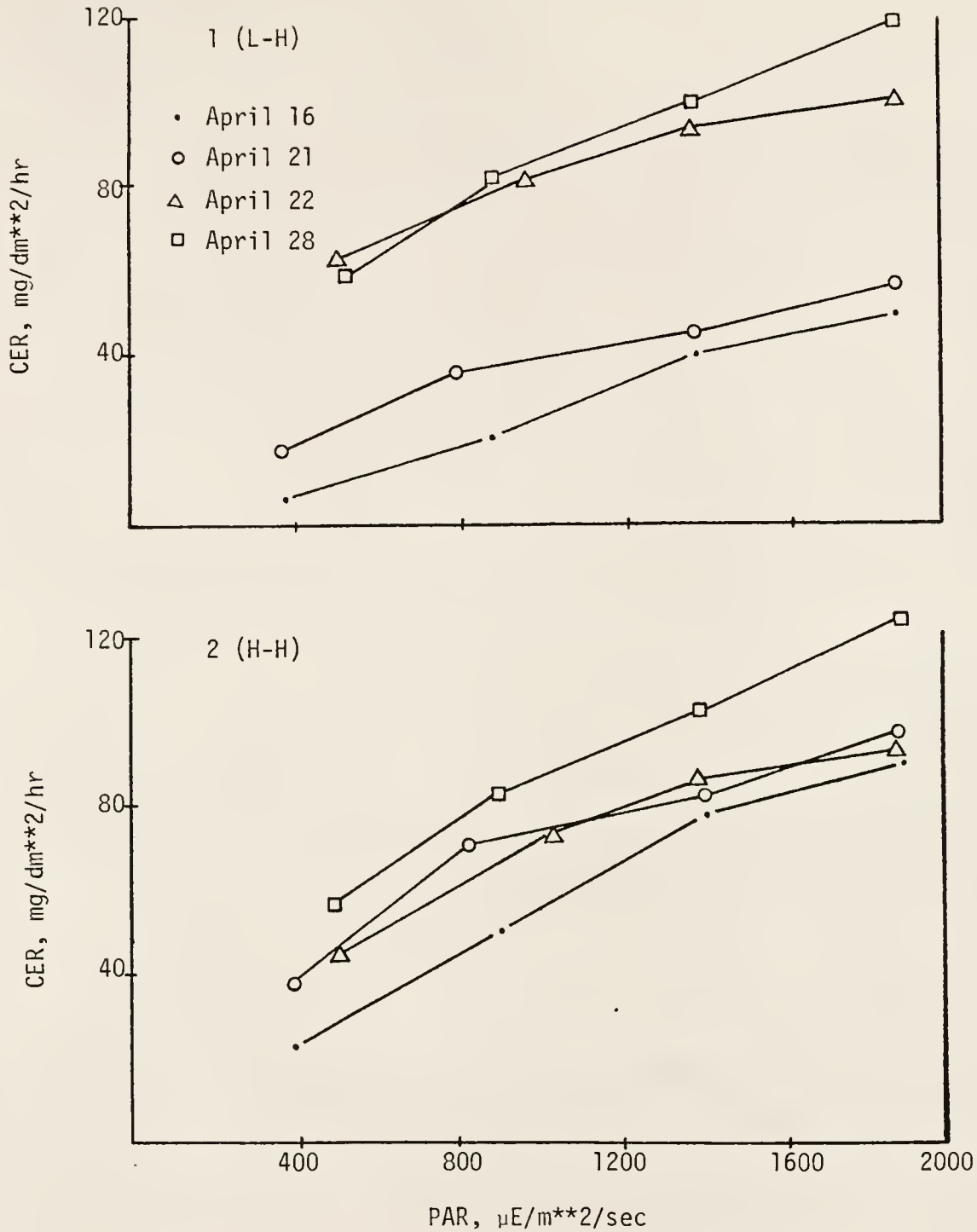


Figure 30. Photosynthetic light response curves; Chambers 1 (Low-High) and 2 (High-High). Quantum flux densities (PAR) are measured in microEinsteins/ m^2 land area/sec. CO_2 exchange rates (CER) are measured in mg CO_2 fixed/ dm^2 land area/hour.

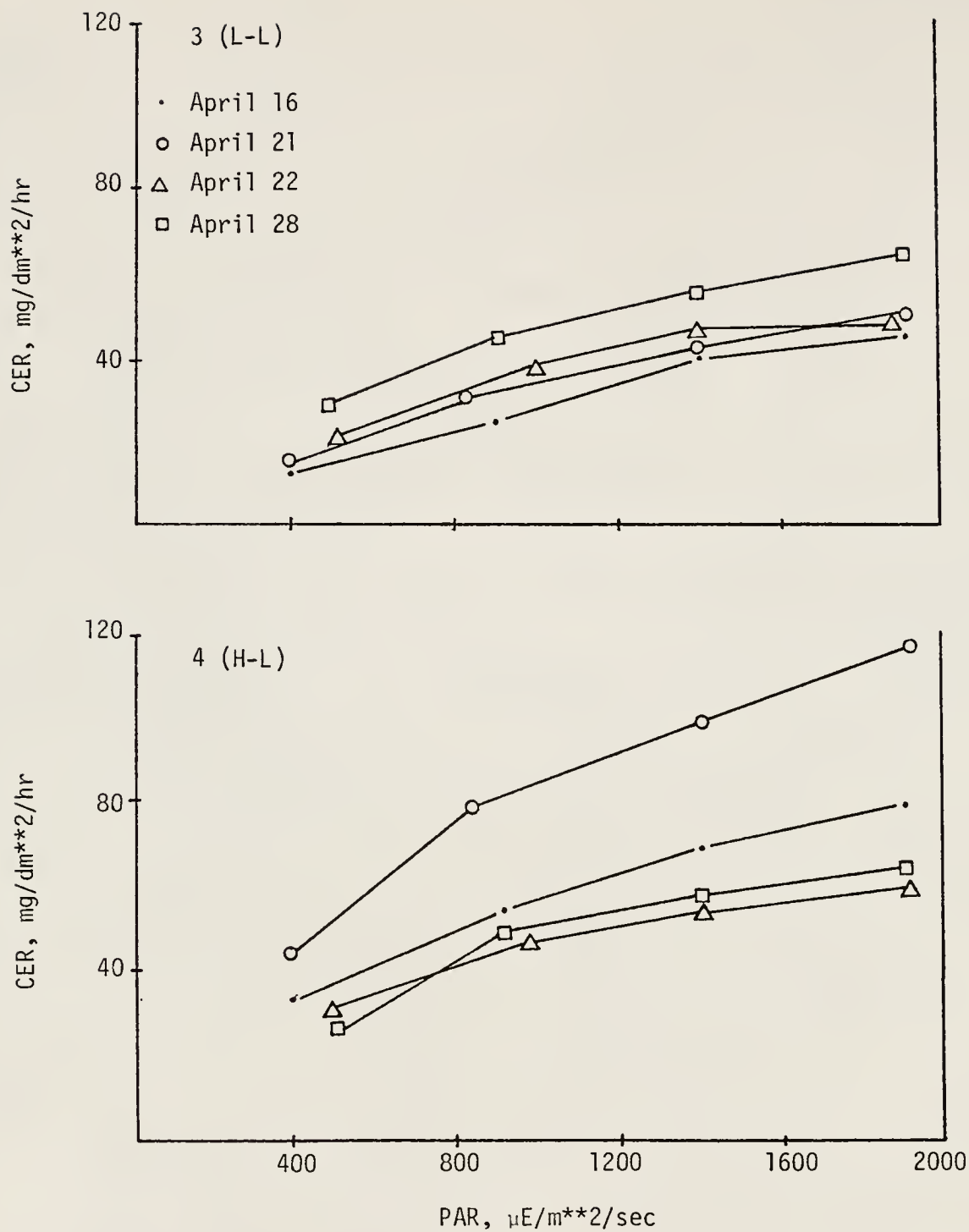


Figure 31. Photosynthetic light response curves; Chambers 3 (Low-Low) and 4 (High-Low). PAR and CER are on a land area basis.

Chamber 3 (L-L) response, given in Figure (31), shows a steady increase with time, consistent with the constant CO_2 levels (320 vpm) maintained. Chamber 4 (H-L) response decreased dramatically from its high point on April 21 when CO_2 level was reduced on April 22. By April 28, the response curves of chambers 3 (L-L) and 4 (H-L) were very similar.

Dark respiration measurements taken at night corroborate and supplement the daytime net photosynthesis measurements. A comparison of each chamber's dark respiration rates on selected dates around the transition is given in Figure (32). Dark respiration is a measure of metabolic activity which presumably increases as more CO_2 is photosynthetically fixed. The graphs in Figure (32) show that before the switch, chamber 4 (H-L) with high CO_2 had a significantly higher respiration rate than did chamber 1 (L-H); after the switch this was reversed completely.

Transpiration

Another dramatic difference between high and low CO_2 concentrations was in transpiration rates. Chambers with elevated CO_2 levels had lower rates than the low CO_2 chambers. When $[\text{CO}_2]$ was switched, the transpiration rates adjusted to the new levels immediately. A time course of rates is given in Figure (33). Chambers 1 (L-H) and 4 (H-L) can be seen to rapidly switch on April 22, the transition date. This is significant to the carbon balance, since transpiration is a direct indicator of stomatal resistance to gas diffusion. Thus, reduced transpiration rates in high CO_2 chambers indicate that the higher CO_2 gradient is somewhat counter-balanced by increased resistance to the CO_2 flux. This response has a beneficial side effect. High CO_2 chambers have

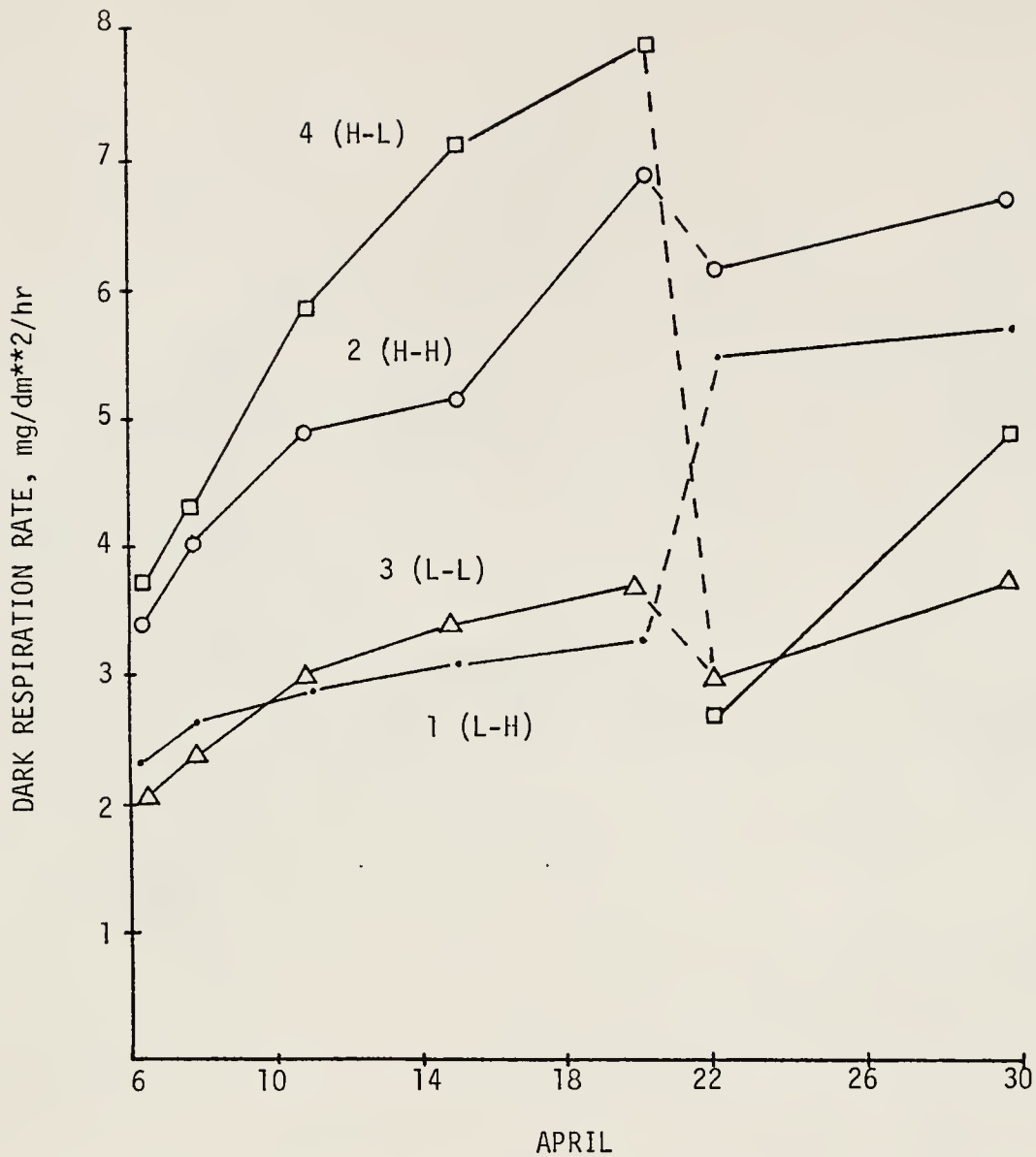


Figure 32. Time courses of dark respiration rates. Average measured pre-dawn respiration rates for each chamber from April 6 to April 30. CO_2 concentrations were switched in chambers 1 (L-H) and 4 (H-L) on April 22. Rates are measured in $\text{mg CO}_2 \text{ evolved/dm}^2 \text{ land area/hour}$.

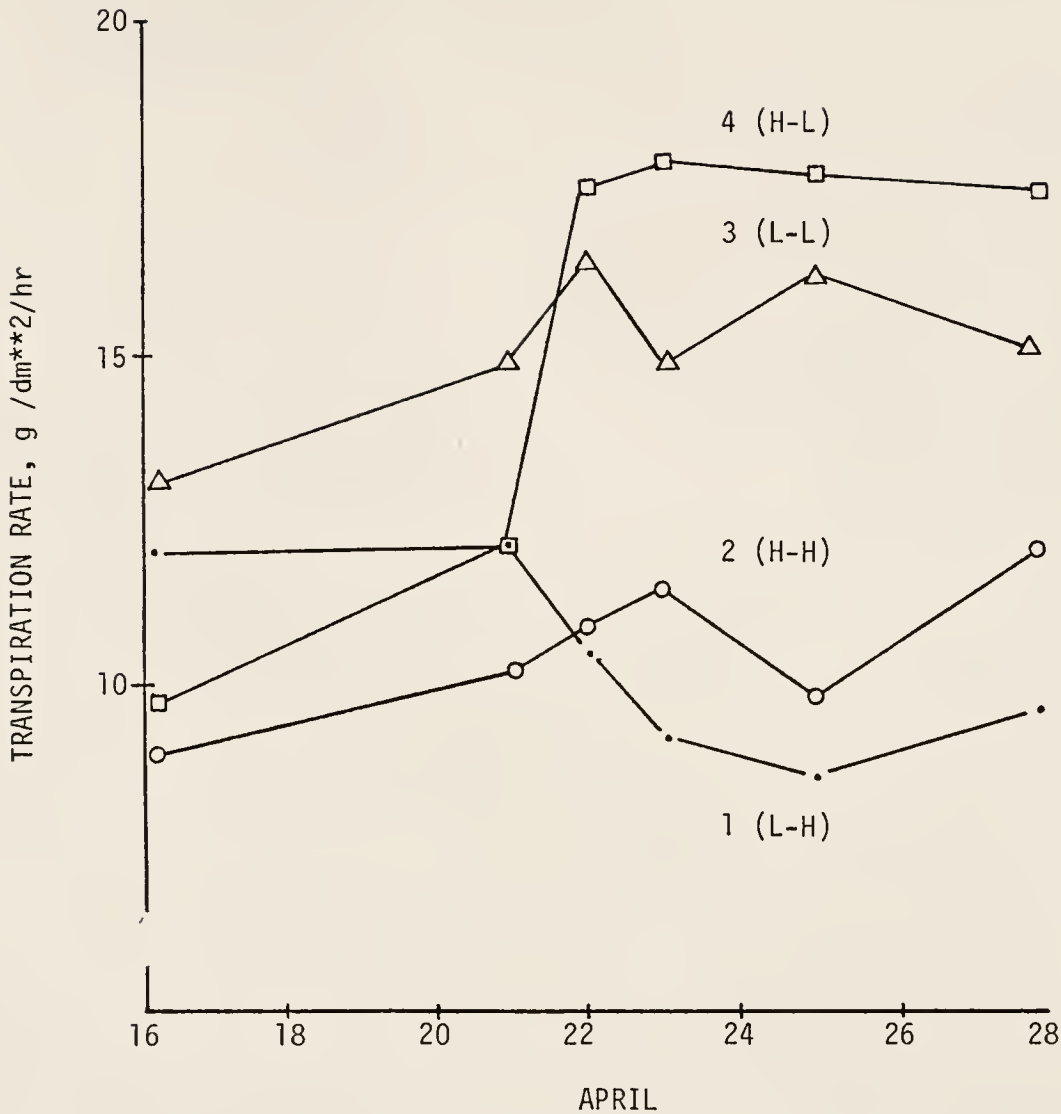


Figure 33. Time courses of transpiration rates. Measured midday transpiration rates from April 16 through April 28. CO₂ concentrations were switched in chambers 1 (L-H) and 4 (H-L) on April 22. Rates are measured in g of H₂O/dm² land area/hour.

relatively greater photosynthetic rates than low CO₂ chambers and they have lower water requirements. To demonstrate this, a time course of water use efficiency is given in Figure (34).

Plant Growth Parameters

Measurements of leaf area between March 20 and April 9 showed that leaves in high CO₂ chambers expanded more rapidly than those in low CO₂ chambers. In the case of the 2nd trifoliolate, leaves in chambers 2 (H-H) and 4 (H-L) took 6 days to fully expand, while leaves in 1 (L-H) and 3 (L-L) took 8 days. All of the leaves grew to about the same final area. For the 4th trifoliolate, leaves in chambers 2 (H-H) and 4 (H-L) again expanded more rapidly, but their final area was smaller than the low CO₂ chamber leaves after 12 days of growth. These results are shown graphically in Figure (35).

Total canopy leaf area index (LAI) has been estimated, based on vertical leaf distribution data from whole plant samples taken throughout the experiment. The time course of LAI for each of the four chambers is plotted in Figure (36). The results clearly show that high CO₂ chambers had significantly greater leaf area per unit of land area (LAI) than did the low CO₂ chambers. After the switch in CO₂ levels, the leaf area in chamber 1 (L-H) continued to increase while chamber 4 (H-L) began to decline. By May 3, chamber 1 (L-H) had a greater LAI than chamber 4 (H-L).

The leaf area analysis can be put on a leaf mass basis using the vertical distribution of specific leaf weights obtained in the whole plant samplings. Results are given in Figure (37). As before, the plants in low CO₂ chambers had significantly less leaf mass than the plants in high CO₂ chambers before the switch. Also, as with leaf area,

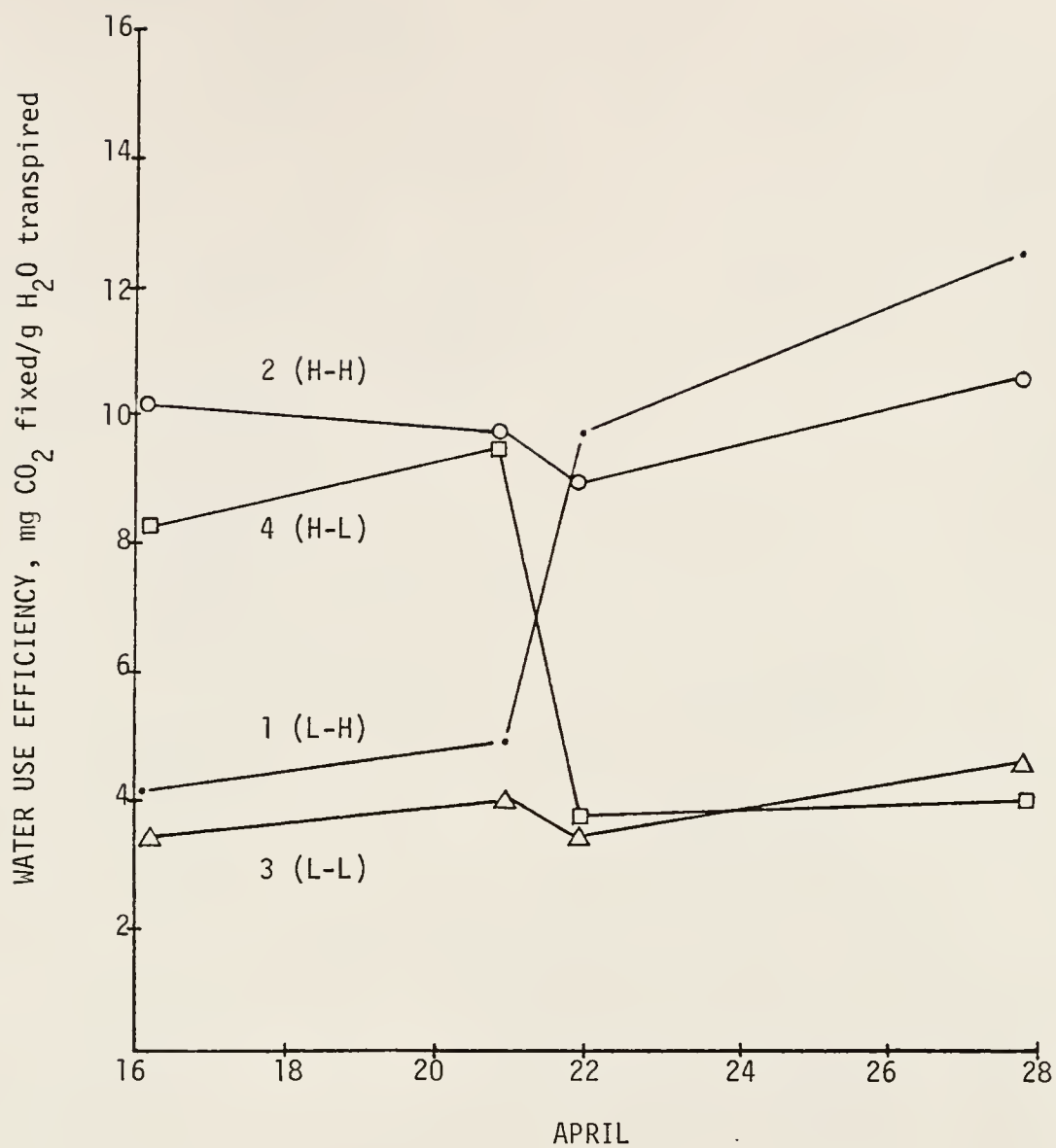


Figure 34. Time courses of water use efficiency. Measurements were taken around noon on sunny days. The CO₂ levels were switched on April 22 at 7:00 a.m.

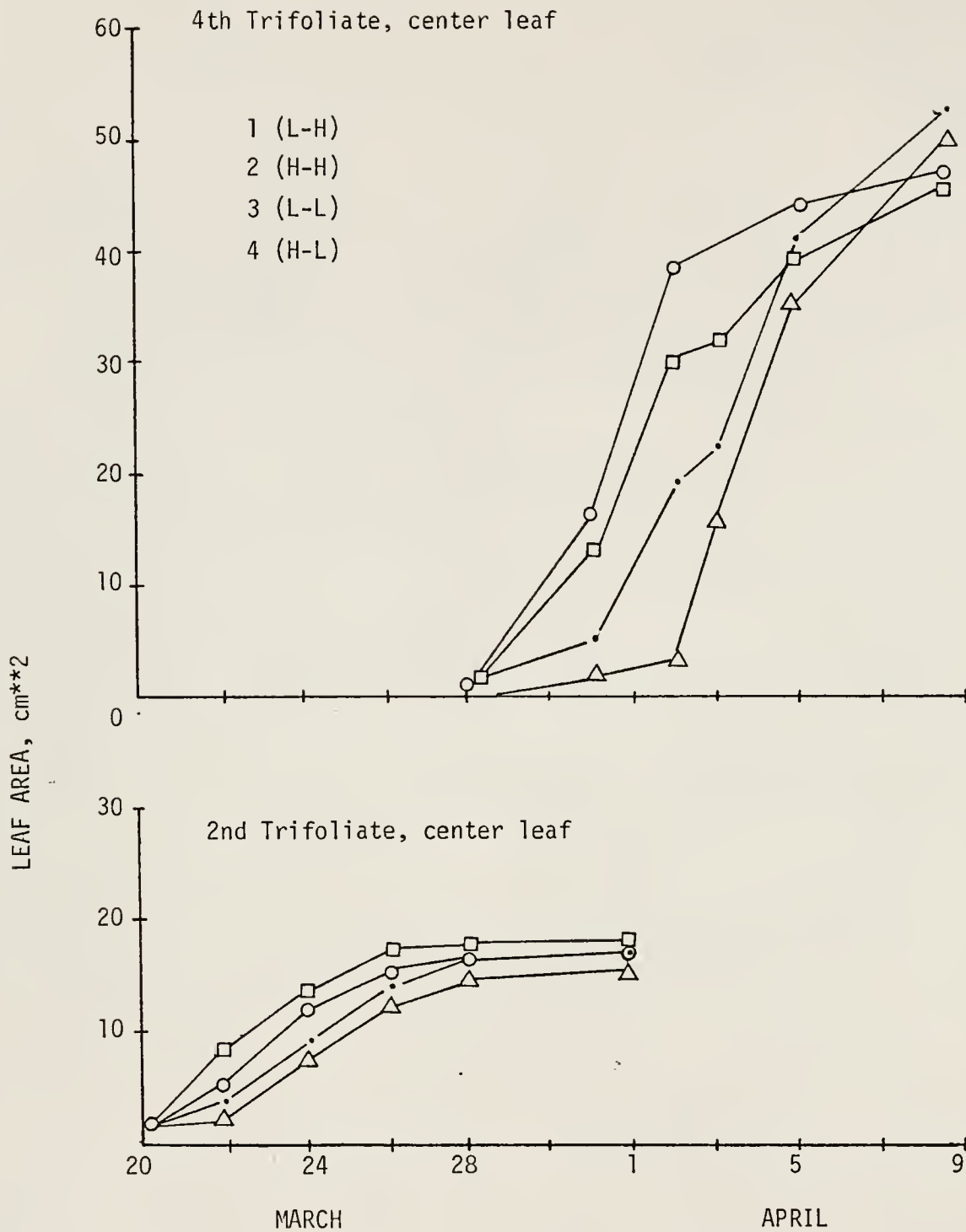


Figure 35. Time courses of individual leaf areas. The central leaf in each trifoliate of four plants in each chamber was measured for length and width. Values for leaf area are average values.

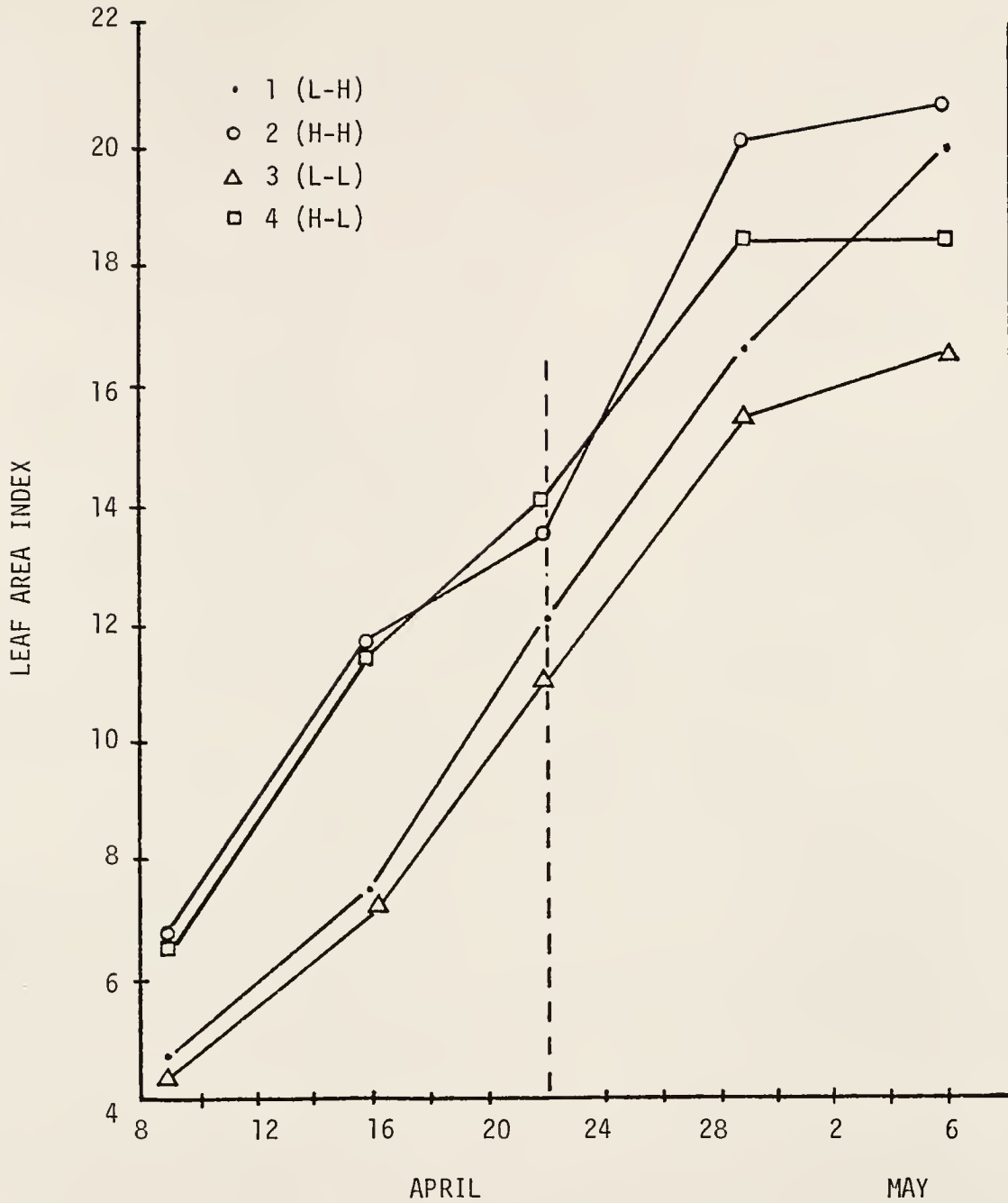


Figure 36. Time courses of leaf area index. LAI measurements are of canopy leaf area divided by land area for each chamber from April 9 through May 6. Note that LAI measurements start at 4. The large values for LAI resulted from light border effects which allowed crop surface areas of 3m^2 to be exposed to full sunlight for 1m^2 of ground area.

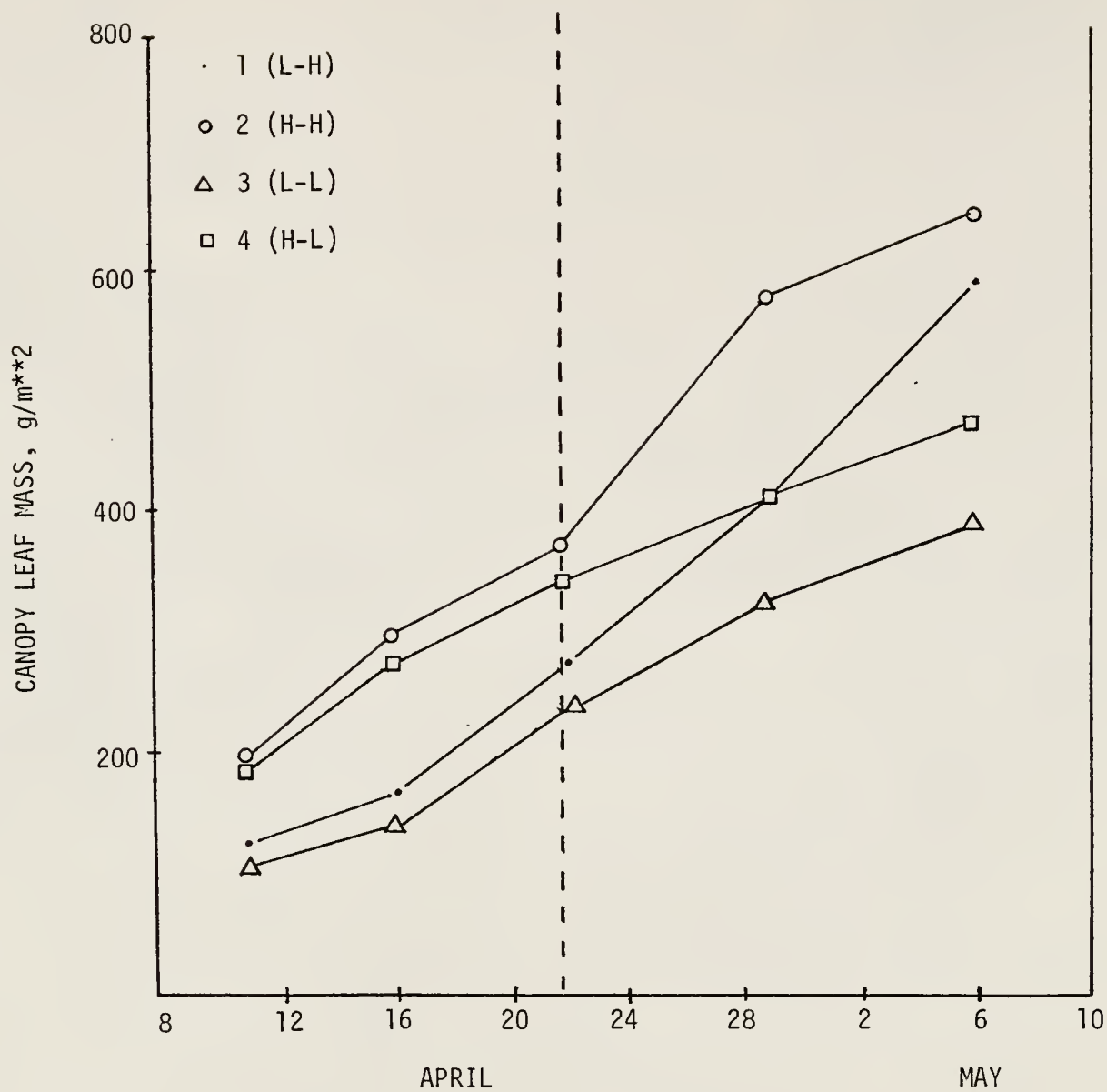


Figure 37. Time courses of canopy leaf mass. Estimated total canopy leaf mass for each chamber from April 11 through May 6. Leaf mass is given on a land area basis.

the leaf mass of chamber 1 (L-H) increased and surpassed the leaf mass of chamber 4 (H-L) after the transition. Overall, the leaf area index and leaf mass time courses in Figures (36) and (37) appear rather similar; however, there are distinctions. First, the leaf mass of chamber 1 (L-H) seems to catch up with chamber 4 (H-L) on April 30, while the canopy leaf areas are not equal until May 3. Furthermore, the final difference in leaf areas gives chamber 1 (L-H) about 10% greater area, while the estimated leaf mass difference gives chamber 1 (L-H) more than 20% greater mass than chamber 4 (H-L). These estimates are supported by the final values for above ground biomass, harvested on May 10. Values are listed in Table (14).

Variations in Photosynthetic Rate

As mentioned before, the daytime course of photosynthetic rates given in Figures (28) and (29) appear to be somewhat skewed with respect to the daytime course of solar radiation, such that photosynthetic rates reach a maximum before the solar noon maximum at approximately 12:30 EST. The implication is that photosynthetic rates in the morning were higher than in the afternoon. For a more detailed consideration, morning and afternoon data were used to generate separate photosynthetic light response curves. In low light curves can be fitted using simple linear regression techniques since response is linear. Typical results are given in Figure (38), in which morning and afternoon regression lines for chambers 1 (L-H) and 4 (H-L) are compared. The data used to generate these graphs are listed in Table (15).

The results show that for chamber 1 (L-H), afternoon responses are lower than the corresponding morning responses, particularly on April 22, the day following transition. The responses in chamber 4 (H-L) are just

Table 14. Final above ground biomass.

	1(L-H)	2(H-H)	Chamber 3(L-L)	4(H-L)
Mass, g.	863.	923.	730.	862.

Note: Values for total above ground dry weight are given. Biomass includes stems, branches and leaves. Plants were harvested on May 10.

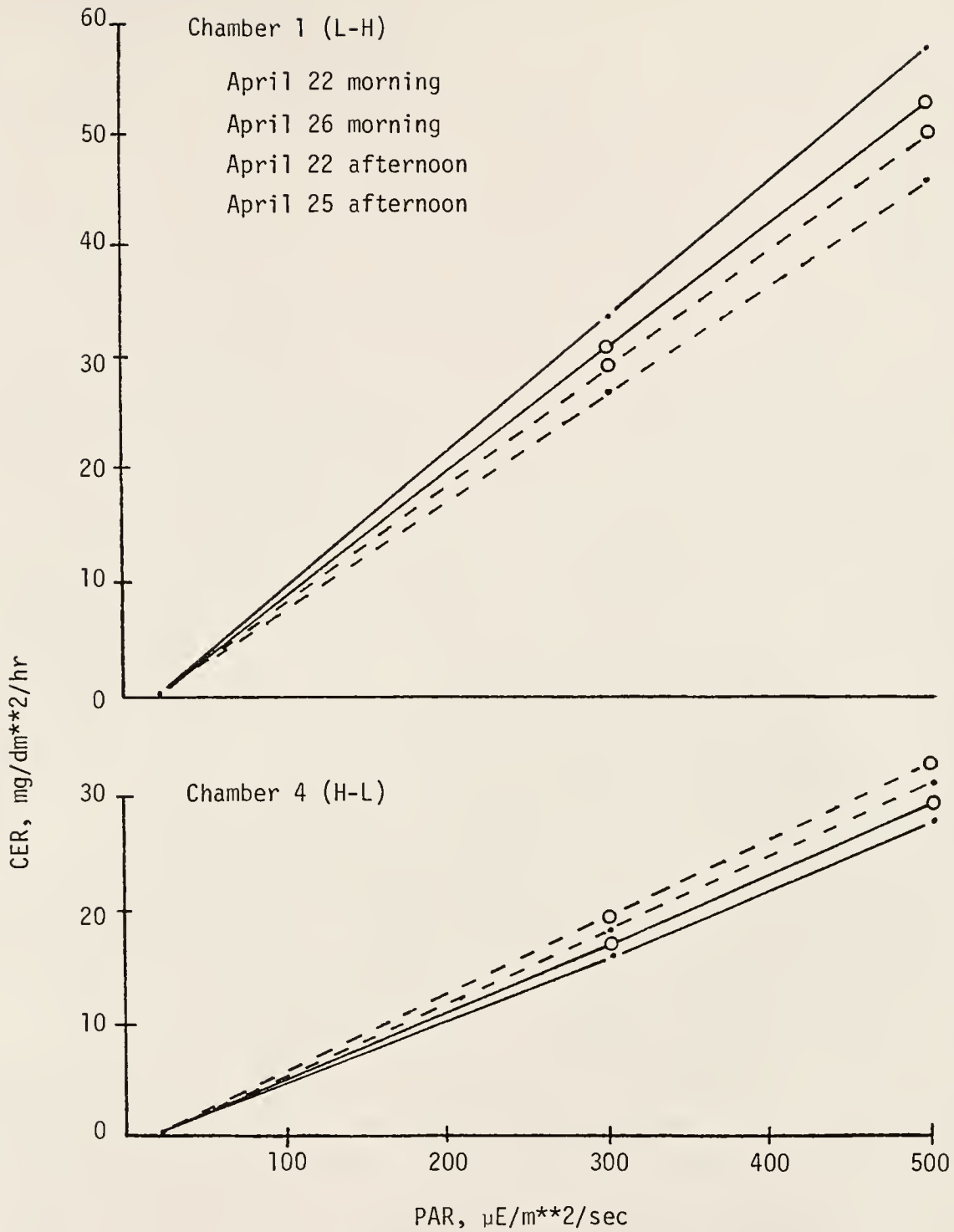


Figure 38. Morning and afternoon photosynthetic light response; Chambers 1 (L-H) and 4 (H-L). Curves result from data fitted by linear regression techniques.

Table 15. Morning-afternoon photosynthetic data.

Date	CHAMBER							
	1(L-H)				4(H-L)			
	Morning		Afternoon		Morning		Afternoon	
	PAR	CER	PAR	CER	PAR	CER	PAR	CER
April 22	426.	56.3	324.	28.2	478.	27.7	332.	20.0
	459.	57.7	353.	32.0	506.	28.4	360.	22.2
	484.	57.9	381.	34.0	536.	30.8	390.	23.8
	547.	63.0	422.	37.9	587.	33.4	431.	25.6
	599.	65.0	458.	40.5	637.	35.9	469.	27.7
	0.	-3.4	0.	-3.4	0.	-3.2	0.	-3.2
April 26/25	338.	39.0	304.	34.4	330.	22.0	314.	23.0
	368.	42.0	367.	41.1	360.	24.0	377.	27.7
	397.	46.3	388.	42.0	389.	24.5	387.	28.0
	444.	52.0	408.	43.9	432.	26.6	423.	30.7
	491.	55.4	450.	48.2	481.	27.7	465.	33.0
	0.	-3.7	0.	-3.7	0.	-3.5	0.	-3.5

Note: These data were analyzed with linear regression techniques to generate the lines in Figure (38). Values for PAR are given in $\mu\text{E}/\text{m}^2/\text{hr}$ and for CER in mg CO_2 fixed/ dm^2 ground area/hr.

the opposite; afternoon regression lines are steeper than the corresponding morning lines.

As a first indicator of the reliability of these regression lines, their correlation coefficients have been calculated. They are listed along with other regression data in Tables (16) and (17). The lowest correlation coefficient found in either table is 0.981. This suggests that the pattern is more than coincidental scatter. To test the null hypothesis that the morning/afternoon pairs could be considered estimates of a single true photosynthetic light response curve, a Student's t-statistic has been calculated for each morning/afternoon pair. From the t-statistic and the degrees of freedom for each pair, the level of confidence with which the null hypothesis can be rejected is prescribed. The t-statistic and confidence level for each pair are listed in Tables (16) and (17).

Results in Table (16) show that for chamber 1 (L-H), the difference in morning and afternoon photosynthetic rates is significant on all five dates shown, with a confidence level of at least 90% in every case. The pattern of higher morning rates was unaffected by the CO₂ transition on April 22. Chamber 2 (H-H) also had higher morning photosynthetic rates, as indicated by the positive t-statistics, however, the confidence levels are not as consistently high as in chamber 1 (L-H).

Table (17) shows chamber 3 (L-L) to have a different pattern than chambers 1 (L-H) and 2 (H-H). The t-statistic is negative on four of the five days, indicating that afternoon photosynthetic rates were greater than morning rates. The confidence level in all five cases is excellent, higher than 95% in every case. Chamber 4 (H-L) has both positive and negative t-statistics. Before the transition in CO₂ levels,

Table 16. Regression analysis of photosynthesis data from Chambers 1 (Low-High) and 2 (High-Low).

Morning, Date	CHAMBER									
	1(L-H)					2(H-H)				
	A20	A21	A22	A26	M3	A20	A21	A22	A26	M3
CER @ 300 $\mu\text{E}/\text{m}^2/\text{sec}$	14.6	12.3	34.1	33.4	34.2	41.7	36.9	24.3	29.9	33.4
CER @ 500 $\mu\text{E}/\text{m}^2/\text{sec}$	27.0	22.7	58.1	58.0	60.0	71.5	66.0	44.3	54.7	58.5
Slope	.062	.052	.120	.122	.129	.149	.145	.099	.124	.126
Correlation Coefficient	.999	.999	.992	.998	.994	.990	.999	.993	.997	.998

Table 16. (contd.)

Afternoon, Date	A20	A21	A22	A25	M2	A20	A21	A22	A25	M2
CER @ 300 $\mu\text{E}/\text{m}^2/\text{sec}$	9.5	9.8	25.9	31.8	27.6	32.7	28.1	21.5	30.9	27.9
CER @ 500 $\mu\text{E}/\text{m}^2/\text{sec}$	18.3	18.6	45.4	55.2	49.2	57.9	50.8	40.0	55.4	50.3
Slope	.044	.044	.097	.117	.108	.126	.113	.093	.122	.112
Correlation Coefficient	.998	.998	.999	.998	.999	.992	.996	.998	.998	.999
t-Statistic	7.24	4.46	4.25	1.64	4.84	1.46	6.94	1.12	.32	2.95
Confidence Level	99%	99%	99%	90%	99%	90%	99%	85%	50%	99%

Note: Morning and afternoon photosynthetic response data under low PAR have been fitted by linear regression. The hypothesis that morning and afternoon curves are both deviations from the same "true" response curve has been tested. Dates are abbreviated: A for April and M for May.

Table 17. Regression analysis of photosynthesis data from Chambers 3 (Low-Low) and 4 (High-Low).

Morning, Date	CHAMBER									
	3(L-L)			4(H-L)						
	A20	A21	A22	A26	M3	A20	A21	A22	A26	M3
CER @ 300 $\mu\text{E}/\text{m}^2/\text{sec}$	14.7	9.5	11.6	11.8	11.9	50.4	42.0	15.7	18.0	20.2
CER @ 500 $\mu\text{E}/\text{m}^2/\text{sec}$	27.1	18.3	21.6	22.3	22.8	83.8	75.5	28.2	31.7	36.0
Slope	.062	.044	.050	.052	.055	.166	.153	.062	.068	.079
Correlation Coefficient	.999	.998	.998	.996	.992	.982	.997	.999	.988	.997

Table 17. (contd.)

Afternoon, Date	A20	A21	A22	A25	M2	A20	A21	A22	A25	M2
CER @ 300 $\mu\text{E}/\text{m}^2/\text{sec}$	13.4	12.9	13.8	16.4	14.8	46.7	34.8	17.3	20.9	20.9
CER @ 500 $\mu\text{E}/\text{m}^2/\text{sec}$	24.3	23.9	25.2	29.5	27.3	78.9	60.7	30.7	37.0	37.5
Slope	.054	.055	.057	.065	.063	.161	.128	.067	.080	.083
Correlation Coefficient	.996	.999	.998	.999	.999	.981	.993	.998	.998	.997
t-Statistic	2.94	-4.11	-2.62	-4.17	-1.62	.51	5.06	-2.32	-3.69	-1.03
Confidence Level	99%	99%	99%	99%	95%	50%	99%	99%	99%	80%

Note: Morning and afternoon photosynthetic response data under low PAR have been fitted by linear regression. The hypothesis that morning and afternoon curves are both deviations from a single "true" response curve has been tested. Dates are abbreviated: A for April and M for May.

chamber 4 (H-L) morning photosynthetic rates were higher, while after the switch, afternoon rates were greater.

In the mechanism proposed in the model development section, photosynthetic inhibition correlates directly with increasing carbohydrate levels. Since carbohydrate levels typically increase during the day, the observed afternoon inhibition was expected. However, the photosynthetic enhancement observed in chamber 3 (L-L) and in chamber 4 (H-L) after its switch to low CO_2 was not anticipated.

One possible explanation for the enhanced afternoon rates may be found in variable canopy leaf temperatures. Although air temperature was well controlled at 25°C , the variable energy budget experienced in each canopy must have caused individual leaf temperatures to vary. Early in the morning leaf temperatures were probably near 25°C , but late afternoon leaf temperatures may have been higher than 25°C . If so, photosynthetic rates may have been enhanced. In chambers 1 (L-H) and 2 (H-H), as well as chamber 4 (H-L) before the CO_2 transition, the photosynthetic inhibition related to carbohydrates could have overwhelmed any enhancing temperature effects. However, chamber 3 (L-L) may have been particularly vulnerable to temperature effects because photosynthetic rates (and presumably, carbohydrate levels) were low. In chamber 4 (H-L) leaf carbohydrate levels were measured and found to be significantly lowered after CO_2 transition (see Figure 42). Therefore, temperature enhancement could have predominated in these two chambers.

Another interesting feature of Tables (16) and (17) are the time courses of CER at $300 \mu\text{E}/\text{m}^2/\text{sec}$ and at $500 \mu\text{E}/\text{m}^2/\text{sec}$, which show the morning and afternoon response to the CO_2 transition in chambers 1 (L-H) and 4 (H-L) in contrast to the constant chambers 2 (H-H) and

3 (L-L). The values show that each canopy's photosynthetic rate is variable not only morning to afternoon but also from one morning to the next. Figure (39) compares the morning time courses of photosynthetic rates at $500 \mu\text{E}/\text{m}^2/\text{sec}$ for each chamber. Afternoon time courses are graphed in Figure (40). Each figure also has time courses of total integrated daily PAR and minimum daily air temperature.

Aside from the sharp responses to CO_2 transition, the graphs show day to day variability which may result from residual source/sink patterns established during preceding days. For instance, carbohydrate reserves may have been used and not replaced during the cloudy weather on April 19. Therefore, leaf carbohydrate levels may have been unusually low on April 20 causing a relative enhancement of photosynthesis.

Minimum ambient air temperatures may offer another clue to the source/sink patterns. The root zone in each chamber was continuously exposed to ambient air, in addition the canopies were flushed with ambient air every hour for 15 minutes during the night. As a result, the "control chambers" were not perfectly insulated from ambient conditions. Under low temperatures, metabolic processes slow down which conserves carbohydrate levels. On April 21, air temperatures were very low and photosynthesis declined in all chambers.

The underlying mechanism tying these variables together is assumed to relate to carbohydrate levels and associated biochemical process rates. This proposed mechanism is described in detail in the model development and results sections. Briefly, carbohydrates originate in photosynthesizing cells, from which they are exported to heterotrophic cells throughout the plant. When more CO_2 is fixed in the source leaves than is utilized by the plant's distributed sinks, carbohydrates accumulate

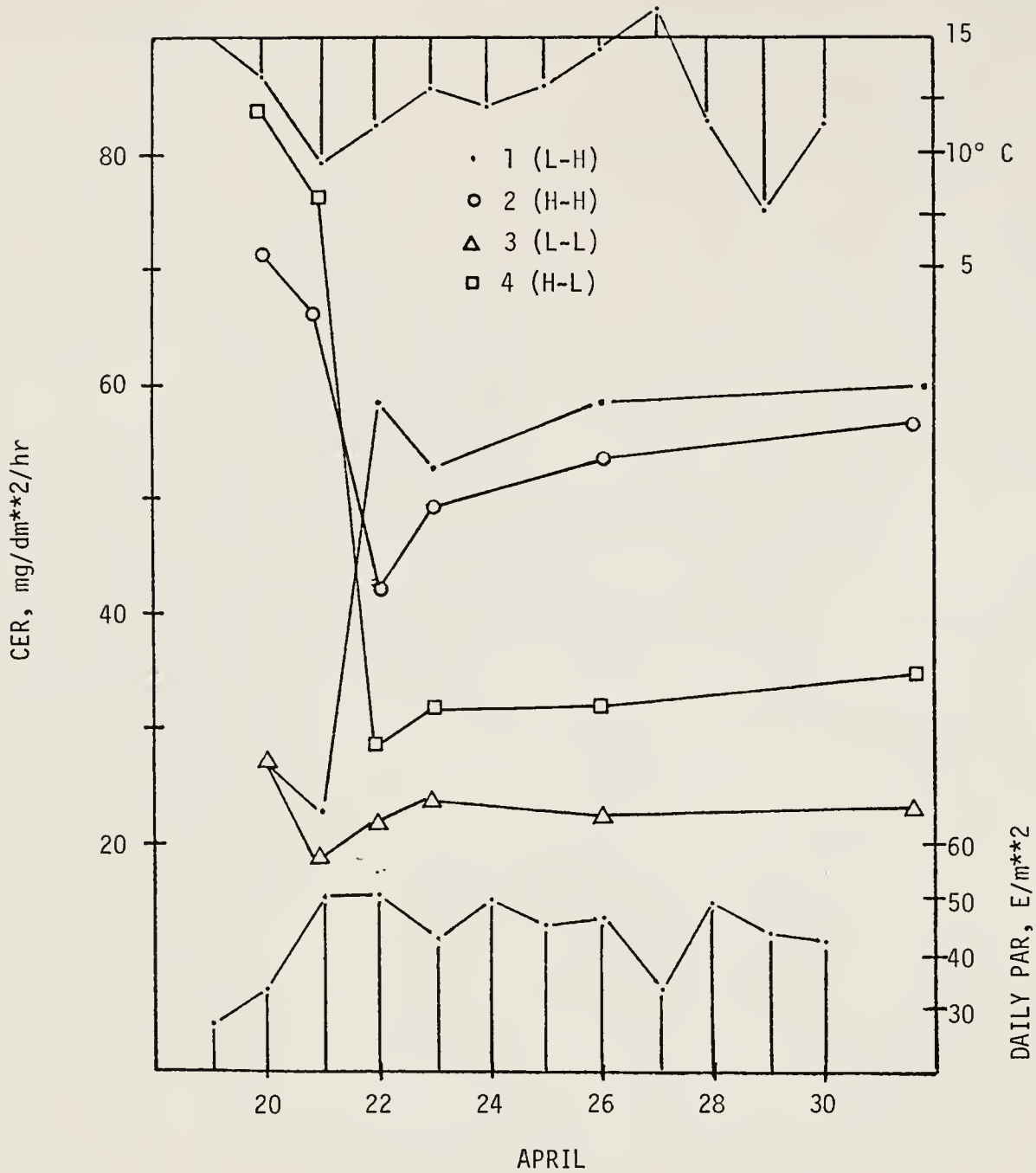


Figure 39. Time courses of morning CER at $500 \mu\text{E}/\text{m}^2/\text{sec}$. Minimum daily temperature values and integrated daily PAR are also given. CER is given in $\text{mg CO}_2 \text{ fixed}/\text{dm}^2 \text{ land area}/\text{hour}$.

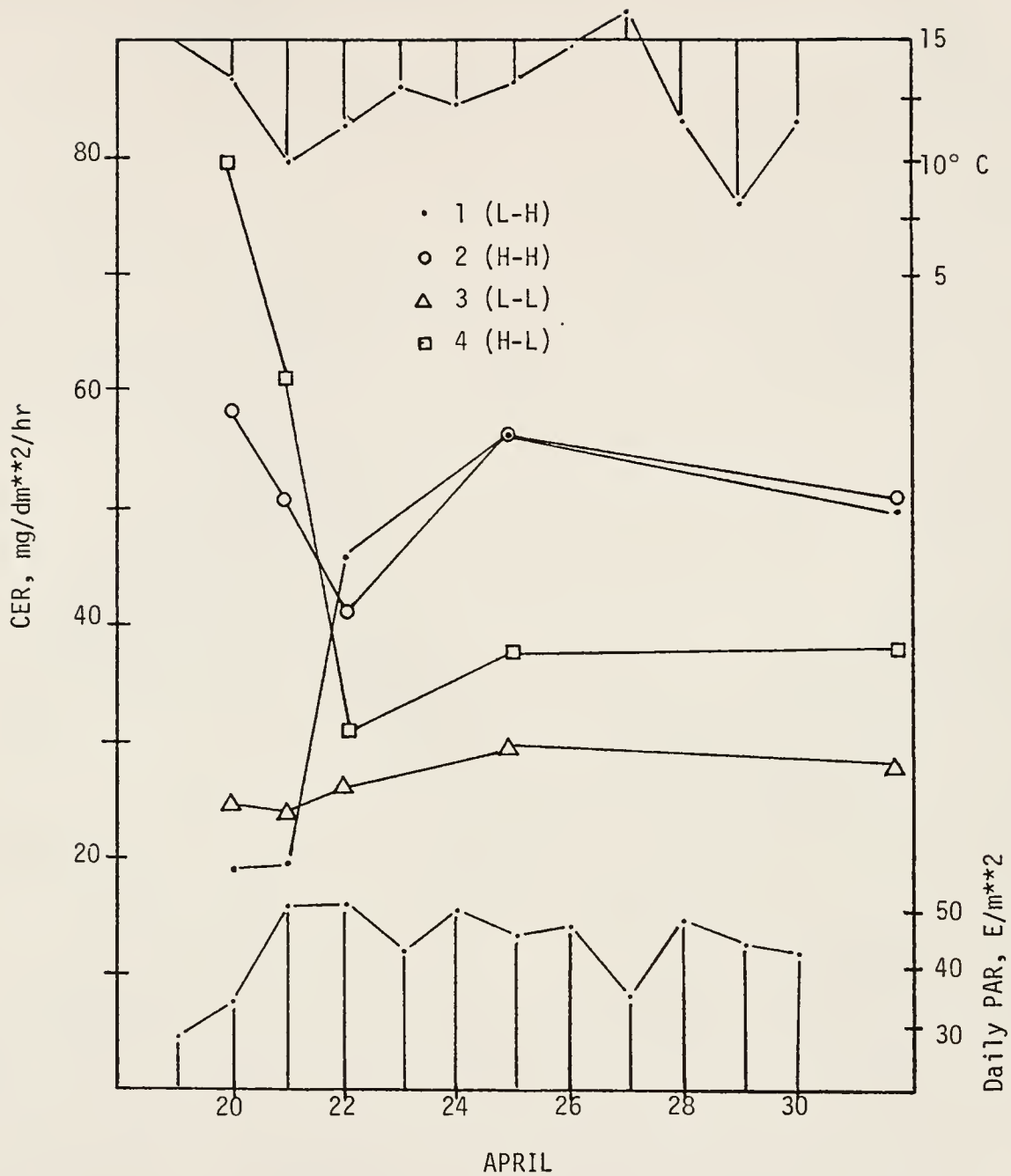


Figure 40. Time courses of afternoon CER at $500 \mu\text{E}/\text{m}^2/\text{sec}$. Minimum daily temperature values and integrated daily PAR are also given. CER is given in $\text{mg CO}_2 \text{ fixed}/\text{dm}^2 \text{ land area}/\text{hour}$.

in the photosynthesizing cells. Associated with the accumulated starch and sucrose levels are feedback effects on the various biochemical cycles which ultimately reduce the rate of carbon fixation. Via this mechanism, the cloudy weather on April 19 may have sufficiently reduced carbohydrate levels to cause enhanced photosynthetic rates on April 20, which caused accumulation of starch and reduced CO_2 uptake on April 21. In addition, the low night time temperatures may have suppressed night time respiration on April 20 and 21, thereby reducing the breakdown of carbohydrates and enhancing accumulation.

In summary, the low photosynthetic rates on April 21 resulted from reduced sink demand caused by the high photosynthetic rates on April 20 (oversupplying carbohydrates) and the low temperature dependent metabolic rates on the night of April 20/21 (reducing carbohydrate consumption).

After the $[\text{CO}_2]$ transition, the photosynthetic rates in chamber 2 (H-H), 3 (L-L), and 4 (H-L) increased from the morning of April 22 to April 23; however, chamber 1 (L-H) declined. This is consistent with the mechanism briefly outlined above. On April 22, chamber 1 (L-H) was exposed for the first time to a high CO_2 environment and photosynthetic rate soared, as did carbohydrate production. However, the canopy sinks were still characteristic of the low CO_2 environment and were not able to utilize the amount of photosynthate that the sources provided. It is assumed that starch accumulated and was still present on April 23 in levels high enough to cause some inhibition. After April 23 inhibition declined as the plants, which were in the vegetative phase, rapidly increased sink strength as shown in the leaf mass measurements.

Physiological Responses

Specific leaf weights (SLW) were measured on selected dates for chambers 1 (L-H) and 4 (H-L). Results are given in Table (18) and

Table 18. Specific leaf weight measurements.

Date	Morning	CHAMBER			
	Time	1(L-H)	2(H-H)	3(L-L)	4(H-L)
A11	9 AM	2.14	2.66	2.07	2.30
A16	8 AM	2.22	2.13	2.02	2.06
A17	9 AM	2.32	2.71	2.11	2.35
A21	9 AM	2.44	3.01	2.24	2.38
A22	8 AM	2.18	2.65	2.54	2.79
A29	9 AM	2.77	3.14	2.37	2.47
M6	9 AM	2.80	2.98	2.61	2.62
Afternoon					
A11	5 PM	2.42	2.45	2.17	2.56
A16	5 PM	2.51	2.75	2.38	3.15
A21	3 PM	2.78	3.87	2.90	3.02
A22	4 PM	3.20	4.00	2.52	3.51
A29	1 PM	2.81	2.99	2.65	3.79
M6	4 PM	3.76	4.15	3.59	3.81

Note: Morning and afternoon specific leaf weights from fully expanded and sunlit leaves. Specific leaf weight, mg/cm². Dates are abbreviated: A for April and M for May.

Figure (41). On April 16, SLW increased more rapidly in chamber 4 (H-L) than in chamber 1 (L-H). On April 22, although the specific leaf weights measured in chamber 4 (H-L) were greater than in chamber 1 (L-H), the rate of increase and absolute increase were greater in chamber 1 (L-H). By May 6, chamber 1 (L-H) had higher absolute values. The variability in measurements, most notable on May 6, may have been caused by sampling procedure. All measurements were made on fully expanded exposed leaves; however, the samples were taken alternately from the east and west ends of the chambers. This procedure may have introduced some variability into leaf weights due to canopy architecture.

Variations in SLW are generally found to correspond somewhat to leaf carbohydrate levels. Samples from chamber 1 (L-H) and 4 (H-L) were analyzed for total soluble carbohydrate (primarily, sucrose and starch). The results in Table (19) show specific leaf weights and carbohydrate levels to be closely dependent. This is shown graphically in Figure (42). The pattern is consistent for all dates in chamber 1 (L-H) and on April 22, in chamber 4 (H-L). On April 29 and May 2, the SLW/carbohydrate pattern seems to break down for chamber 4 (H-L) and levels are uniformly low. This probably reflects a continuing source/sink imbalance. On April 22, chamber 4 (H-L) was switched from a high to a low CO₂ environment and source strength declined while sink demand remained high. On April 29 and May 2, the sink demand remained high enough to keep leaf carbohydrate levels low.

Leaf protein level is closely tied to total leaf nitrogen level. Hence, total nitrogen was measured as an indicator of enzyme proteins. Vertical distribution of total nitrogen was measured on dates when whole plant samples were taken. Results are listed in Table (20). The data are separated into vertical groups, 4 and 5 refer to the fourth and

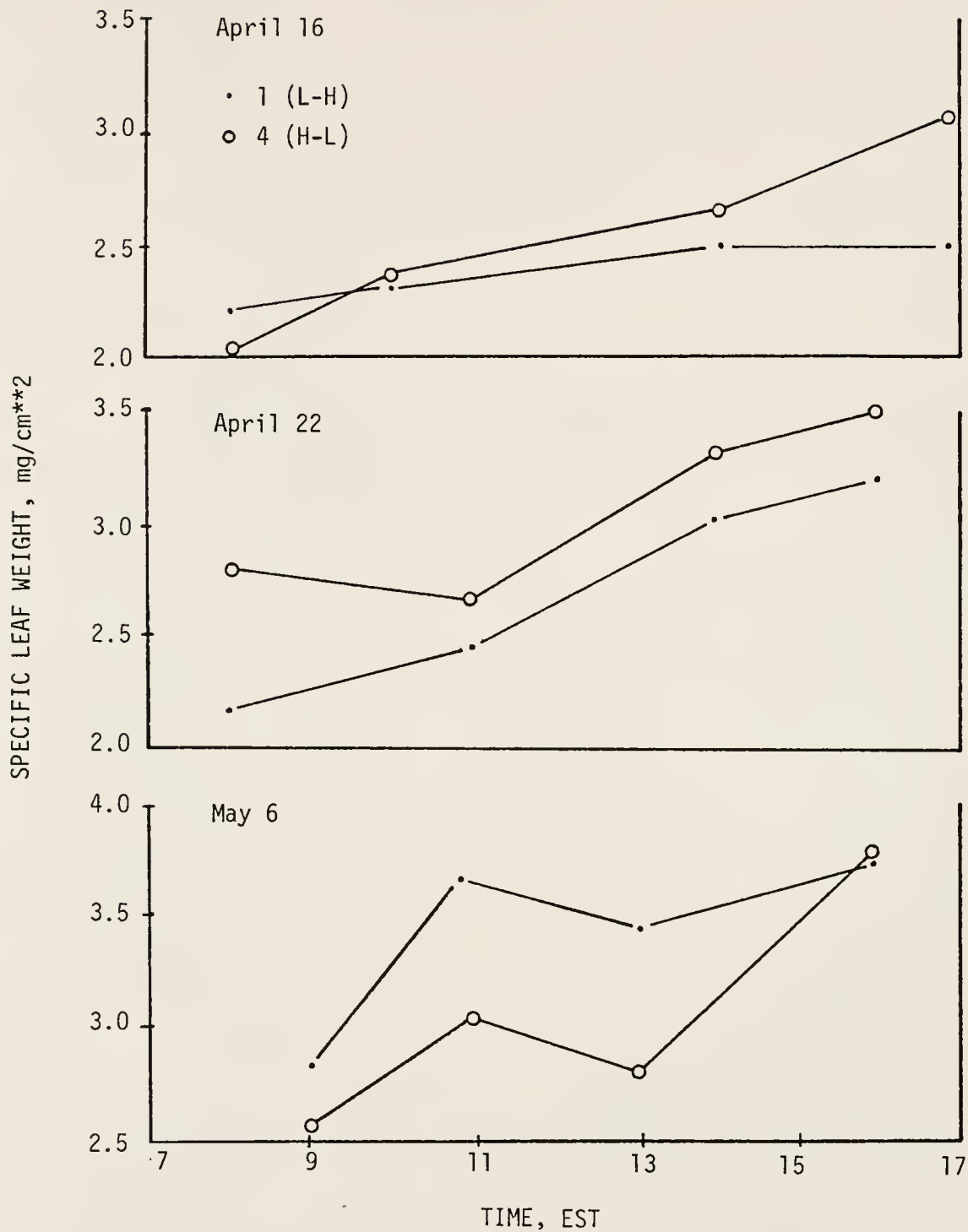


Figure 41. Diurnal specific leaf weights; Chambers 1 (Low-High) and 4 (High-Low). Measurements were taken on exposed fully expanded leaves. Specific leaf weight (SLW) units are mg of leaf mass/ cm^2 of leaf area.

Table 19. Comparison of specific leaf weight and soluble carbohydrates in Chambers 1 (L-H) and 4 (H-L).

Date	Node	Chamber			
		1 (L-H)		4 (H-L)	
		SLW	% SC	SLW	% SC
A22	5	2.26	12.27	1.69	10.95
	7	2.07	9.63	2.67	15.72
	9	2.45	14.63	2.73	11.91
A29	5	1.81	10.73	1.98	4.79
	7	1.74	9.87	2.08	5.89
	9	1.57	6.47	2.63	6.07
M2	7	2.41	13.14	2.97	7.58
	9	2.67	14.19	2.63	6.93
	11	2.87	14.56	2.29	7.02

Note: Measurements were made on leaves from whole plant sample taken at 11:00 EST on sunny days. Node refers to the vertical distribution of the leaves sampled. Nodes were counted from the ground upward. Specific leaf weight is in units of mg/cm² and soluble carbohydrate (SC) values are given as % of dry weight.

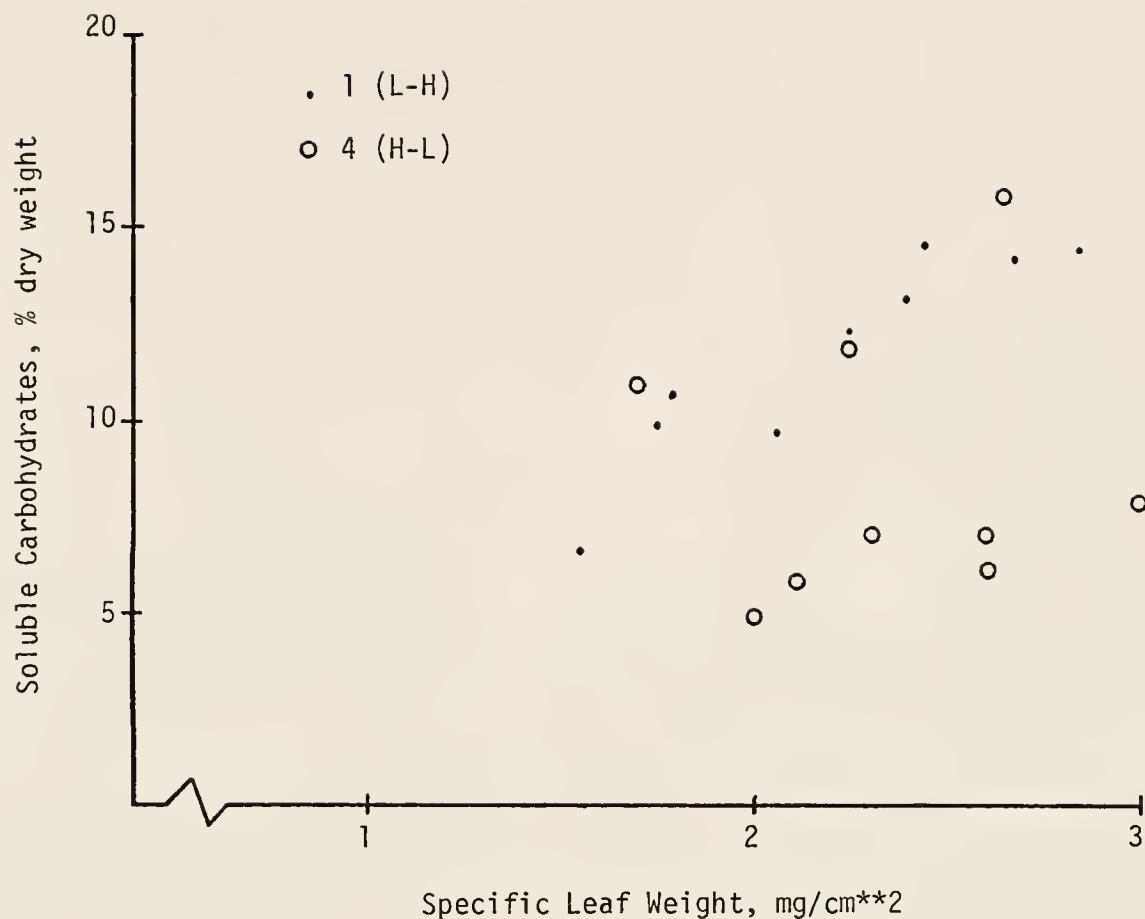


Figure 42. Comparison of carbohydrate levels and specific leaf weight. Graph compares measurements at 11:00 EST on April 22, April 29 and May 2 taken in chambers 1 (L-H) and 4 (H-L). The switch in CO₂ level occurred on April 22. The values in the lower right corner are for chamber 4 (H-L) on April 29 and May 2 several days after the CO₂ transition.

Table 20. Vertical distribution of leaf nitrogen.

Node	Date	Nitrogen/Leaf Mass						Total Nitrogen			
		Chamber						Chamber			
		1 (L-H)	2 (H-H)	3 (L-L)	4 (H-L)	1 (L-H)	2 (H-H)	3 (L-L)	4 (H-L)	3 (L-L)	4 (H-L)
4,5	A22	5.10	4.85	5.17	4.48	4.42	4.85	4.20	4.56		
	A29	4.47	3.40	4.48	4.16	3.71	3.75	3.49	4.07		
	M6	3.36	3.80	4.32	3.89	2.71	5.36	3.29	3.70		
6,7	A22	5.42	4.63	5.37	4.56	5.98	6.07	5.70	5.49		
	A29	5.03	4.89	5.20	4.34	5.31	6.15	5.29	5.11		
	M6	3.86	6.59	4.43	4.39	4.07	8.29	4.51	5.16		
8,9	A22	5.63	3.60	5.78	5.57	4.76	3.18	3.47	6.34		
	A29	5.88	4.95	5.76	5.52	9.09	9.71	7.72	8.83		
	M6	4.56	7.40	5.26	5.15	6.93	9.33	5.56	7.93		
10,11	A29	4.89	5.15	6.09	5.67	4.96	5.91	5.80	6.87		
	M6	4.56	6.08	5.70	5.31	6.56	9.25	4.94	6.33		
Total Canopy	A22					15.1	14.0	13.4	16.4		
	A29					23.1	24.9	22.4	24.9		
	M6					24.4	31.1	24.2	25.8		

Note: Nitrogen/leaf mass values are on a % basis and total nitrogen values are in grams.

fifth trifoliates counting from the ground upward. The measurements are presented for each chamber on both a leaf area and a total canopy basis. Comparing the nitrogen/leaf mass values on April 22, chamber 1 (L-H) levels were clearly higher than chamber 4 (H-L). By May 6, the situation was reversed. This is shown graphically in Figure (43). On a total canopy nitrogen basis this disparity is much less evident. If there was a significant difference, chamber 4 (H-L) had more total nitrogen than chamber 1 (L-H). The relationship between the chambers on a total nitrogen basis is summarized in the last three rows of Table (20). The date for chamber 2 (H-H) cannot be accurately interpreted because of physical damage and pest problems.

Chlorophyll was measured on the same dates from the same whole plant samples as the nitrogen. Values are presented on a leaf area and total canopy basis in Table (21). At the 4,5 nodes total chlorophyll values declined over time, while at the 6,7 nodes the values climbed slightly from April 22 to April 29 and then declined. In nodes 8 and 9, chlorophyll increased sharply in all chambers from April 22 to April 29, then dropped somewhat. The pattern suggests that the chlorophyll levels peaked in higher nodes on later dates, perhaps due to leaf aging. The estimates of total canopy chlorophyll given in the bottom rows show more variability than did nitrogen levels. Total estimated chlorophyll content increased in each chamber during the measurement period.

Inorganic phosphate levels were also measured on whole plant sample dates (Table 22). Unlike chlorophyll and enzyme concentrations, inorganic phosphate levels can fluctuate very quickly in response to changing light levels. Since samples were taken several days apart under variable short term conditions, the phosphate concentrations fluctuated enough to mask any trends that may have been present.

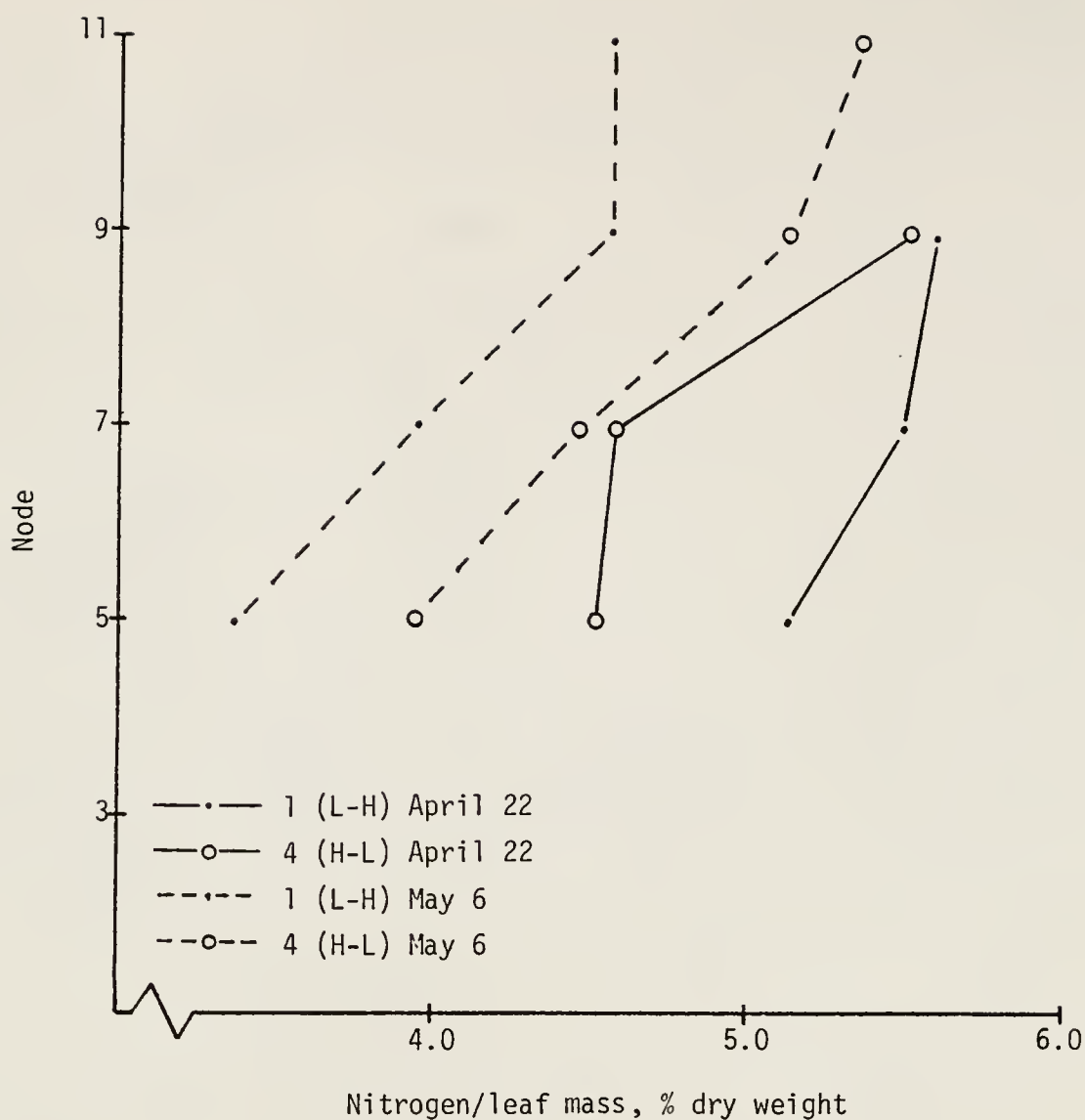


Figure (43). Vertical nitrogen distribution; Chambers 1 (Low-High) and 4 (High-Low). This graph shows the relative switch in leaf nitrogen levels following the $[CO_2]$ transitions in chambers 1 (L-H) and 4 (H-L).

Table 21. Vertical distribution of leaf chlorophyll.

		Chlorophyll/Leaf Area				Total Chlorophyll			
		Chamber				Chamber			
Node	Date	1 (L-H)	2 (H-H)	3 (L-L)	4 (H-L)	1 (L-H)	2 (H-H)	3 (L-L)	4 (H-L)
4,5	A22	1.34	1.38	1.22	1.18	6.38	7.58	5.44	6.62
	A29	1.16	1.28	1.34	1.30	5.30	6.74	5.04	7.00
	M6	1.18	1.00	1.18	1.20	5.26	5.16	4.90	6.32
6,7	A22	1.26	1.52	1.40	1.38	7.64	10.98	8.18	9.16
	A29	1.32	1.76	1.58	1.46	7.68	12.18	8.84	9.46
	M6	1.20	1.26	1.22	1.38	6.82	8.52	6.68	8.60
8,9	A22	1.06	1.12	1.10	1.14	4.94	5.46	3.80	7.14
	A29	1.20	1.52	1.62	1.60	10.68	16.36	11.94	14.10
	M6	1.18	1.36	1.52	1.78	9.86	8.34	8.82	15.08
10,11	M6	1.50	1.00	1.42	1.52	11.88	8.36	6.78	9.94
Total Canopy	A22					18.96	24.02	17.42	22.92
	M6					35.50	31.46	27.20	39.94
	%Δ					87.	31.	56.	74.

Note: Chlorophyll/leaf area values are given in units of mg/cm² and total chlorophyll values are in mg. %Δ indicates the percentage change in total chlorophyll from April 22 to May 6.

Table 22. Vertical distribution of leaf inorganic phosphate.

Node	Date	Phosphate-Leaf Mass			
		Chamber			
		1 (L-H)	2 (H-H)	3 (L-L)	4 (H-L)
4,5	A22	3.16	2.14	4.02	7.95
	A29	1.26	1.30	1.50	1.53
	M6	3.42	3.26	4.69	6.27
6,7	A22	4.62	2.50	3.85	3.47
	A29	2.24	1.53	1.56	1.56
	M6	2.88	5.25	7.73	1.48
8,9	A22	2.44	.92	11.26	2.94
	A29	3.50	2.11	1.95	2.35
	M6	5.29	4.45	5.06	1.40

Note: Phosphate-leaf mass values are in mg/gr dry weight.

SUMMARY AND CONCLUSIONS

In this work photosynthesis has been studied on several distinct levels. At the subcellular level a biochemical photosynthesis model has been developed with very rapid time response. The model describes the CO_2 fixation and carbohydrate synthesis pathways in detail. In addition, it offers a mechanistic explanation of the roles played by starch and sucrose in the regulation of CO_2 uptake. At the whole plant level experiments have been conducted in environmental control chambers. Both short term response and long term adaptation of soybean canopies exposed to differing CO_2 concentrations were monitored. Short term measurements of variables such as CO_2 exchange and specific leaf weight were used to develop diurnal response patterns. In similar fashion, long term measurements such as leaf area and biomass described growth response integrated over time.

The model was evaluated quantitatively by comparing the responses predicted by specific model equations with observed responses as reported in the literature. On this basis the model performs very well. The model was used to successfully predict

- 1) CER as a function of CO_2 concentration,
- 2) overall starch accumulation,
- 3) starch accumulation as a function of inorganic phosphate levels,
- 4) transport of fixed carbon from chloroplast to cytoplasm as a function of cytoplasmic inorganic phosphate levels,

- 5) export of fixed carbon from the cytoplasm as a function of sucrose concentration.

The model was also evaluated qualitatively and found to be generally consistent with the results of whole plant source-sink experiments in the literature. The model's mechanisms were able to account for sink strength feedback regulation of photosynthetic rates. Decreased sucrose export rates caused by reduced sink demand resulted in predicted starch accumulation and decreased photosynthetic rates while increased sink strength resulted in decreased starch levels and enhanced photosynthetic rates.

Results from the experimental work showed definite response to both short term changes in CO_2 concentration and to long term exposure to differing constant CO_2 levels. It was found that soybean canopies grown under elevated CO_2 concentrations had

- 1) higher net photosynthesis rates,
- 2) higher dark respiration rates, and
- 3) lower transpiration rates.

Close analysis of each canopy's response found that photosynthetic light response varied from morning to afternoon and from one morning to the next. High $[\text{CO}_2]$ canopies exhibited afternoon inhibition of net photosynthesis while low $[\text{CO}_2]$ canopies had a mixed response. On a leaf basis the low $[\text{CO}_2]$ canopies seemed to have somewhat lower specific leaf weights and the specific leaf weight increased less diurnally than in the high $[\text{CO}_2]$ canopies. Changes in specific leaf weight were found to correspond to changes in soluble carbohydrate levels.

On a longer time scale high $[\text{CO}_2]$ canopies responded morphologically with

- 1) more rapid leaf expansion,

- 2) increased total leaf area, and
- 3) increased total biomass.

In addition, the total nitrogen levels on a leaf area basis were less in high $[CO_2]$ canopies than in low $[CO_2]$ canopies. When CO_2 levels were switched all of the responses above slowly adapted to the new conditions.

There is an inherent difficulty in quantitatively comparing model and experimental results because of the differences in detail of the modelled state variables and the variables measured in the experiment. The model includes internal variables at the subcellular level whereas experimental measurements were on individual leaf and whole canopy levels. Experimentation at this level was necessary because source-sink balances that directly affect and respond to changing photosynthetic rates occur on a whole plant level. Modelling at this level was necessary because it explicitly describes the mechanism linking source-sink balances and photosynthetic rate regulation. The connection between model and experiment is in recognizing that canopy behavior is the integrated response of each discrete cell in the system. Therefore, even across the large gap in detail, model and experimental results must behave consistently.

Qualitatively the responses predicted by the model and measured in the experiments were consistent. At the most fundamental level increased $[CO_2]$ was shown in the model results to directly promote the carboxylation reaction and to subsequently increase production of carbohydrates. This was confirmed by the short term experimental results which showed enhanced CO_2 uptake rates and long term results which showed enhanced biomass production under high $[CO_2]$.

On a diurnal basis model results showed an increase in starch level and experimental measurements showed a corresponding increase in specific

leaf weight which implied an increase in soluble carbohydrates. In the long term soluble carbohydrate declined when $[CO_2]$ was switched in Chamber 4 (H-L) from a high to a low level. This resulted from the changing source-sink balance.

Canopy 4 (H-L) was grown in high $[CO_2]$ which enhanced its source strength. In tandem with its higher CO_2 fixing capacity the canopy developed a matching sink capacity. When the chamber CO_2 level suddenly declined so did source strength but sink demand capacity remained high. As a result sucrose levels in the photosynthetic tissue remained low because of rapid export rates. Consequently, less starch accumulated and soluble carbohydrate levels declined. The model results showed that high sucrose export rates and reduced cytoplasmic sucrose levels enhanced inorganic phosphate levels which favored export of fixed CO_2 and inhibited starch formation. Thus, the model corresponds to experimental results in predicting lower soluble carbohydrate levels when sink demand is high.

Experimental results also revealed a probable morning-afternoon source-sink relationship. After a night of respiration, morning sink demand was probably high relative to afternoon sink demand. Comparison of relative morning-afternoon net photosynthesis showed significant differences in rates. In Chamber 4 (H-L) with high absolute CO_2 uptake rates, photosynthesis declined significantly in the afternoons. In terms of the model sink requirements declined in the afternoon causing increased sucrose levels and decreased inorganic phosphate levels, which shifted the ATP/ADP ratio and slowed down the Calvin cycle fixation of CO_2 .

After Chamber 4 (H-L) was switched to low CO₂ levels the experimental results showed no afternoon inhibition of photosynthesis. In this case the canopy had excess sink demand capacity and sucrose levels did not increase significantly enough to affect the rate regulating ATP/ADP ratio.

To summarize, net photosynthesis is regulated directly by the specific mix of biochemical substrate concentrations as shown in the model and certain diurnal patterns exist which can affect these interdependent balances as shown in the short term experimental measurements. Finally, the integrated effect of short term patterns over time controls the development of whole canopy source-sink relationships.

In conclusion, the model and experimental results were found to be in good qualitative agreement and to be well described in terms of the source-sink concept. Measurements in the experimental work revealed variations in the photosynthetic light response which have been reported in terms of inhibition or enhancement of "normal" response. The model developed in this work has shown how this dynamic photosynthetic response can be mechanistically regulated by the fluctuating levels of inorganic phosphate at the cellular level.

To further develop this model more complete data sets with emphasis on starch, sucrose, and inorganic phosphate concentrations at frequent time intervals are required. The model will also require a more complete search of the biochemical literature for the various reaction rate constants. Finally, the model should be more realistically linked to the rest of the plant via an export function which responds to sink demand. Once these additions are made the model will be sufficiently complete to be useful in larger scale plant growth models and ultimately in crop simulation models.

In view of steadily increasing global CO_2 levels further experimental work on CO_2 enrichment is certainly justified. Several lines of investigation can be suggested from the results of this research.

Specifically,

1. the canopies grown under elevated CO_2 had a lower nitrogen requirement per unit biomass,
2. the high CO_2 plants were much more water efficient than their low CO_2 counterparts, and
3. canopies adapted their source-sink balances quickly when conditions changed.

These are all topics with important implications in both basic research and applications which require more work.

APPENDIX 1 ABBREVIATIONS LISTING

A	starch accumulation coefficient
AMP	adenosine monophosphate
ADP	adenosine diphosphate
ADP1	chloroplastic ADP
ADP2	cytoplasmic ADP
AREA	chamber ground surface area
ATP	adenosine triphosphate
ATP1	chloroplastic ATP
ATP2	cytoplasmic ATP
B	starch accumulation coefficient
BPGA	1,3-diphosphoglyceric acid
C1	reaction rate constant 1
C2	reaction rate constant 2
C3	reaction rate constant 3
C4	reaction rate constant 4
C5	reaction rate constant 5
CER	CO ₂ exchange rate, net photosynthesis
CH ₂ O	carbohydrate or photosynthate
C ₆ H ₁₂ O ₆	glucose
C ₁₂ H ₂₂ O ₁₁	sucrose
CO ₂	carbon dioxide
[CO ₂]	chloroplastic (stromal) CO ₂ concentration
[CO ₂] _a	ambient CO ₂ concentration
[CO ₂] _d	desired CO ₂ concentration
[CO ₂] _e	final CO ₂ concentration
[CO ₂] _i	intercellular CO ₂ concentration
[CO ₂] _o	initial CO ₂ concentration
[CO ₂] _m	measured CO ₂ concentration
CR	chamber diffusion resistance

DHAP	dihydroxyacetone phosphate
DHAP1	chloroplastic DHAP
DHAP2	cytoplasmic DHAP
DL	diffusional leakage rate
E	enzyme
ECO ₂	enzyme CO ₂ complex
ECO ₂ RuBP	enzyme, CO ₂ , and RuBP complex
EMP	erythrose 4 phosphate
EST	eastern standard time
[EXPORT]	rate of sucrose transport from cytoplasm
FBP	fructose-1,6-phosphate
FMP	fructose-6-phosphate
G	Gibbs' free energy
G°	Standard Gibbs' free energy
GAP	glyceraldehyde-3-phosphate
G1P	glucose-1-phosphate
G6P	glucose-6-phosphate
h	Planck's constant
H-H	high to high [CO ₂]
H-L	high to low [CO ₂]
H ₂ O	water
k ₁ - k ₁₀	process rate constants 1 through 10
Kc	Michaelis Menten constant for CO ₂ process
Kd	M-M constant for DHAP1 transport process
Kd2	M-M constant for DHAP2 conversion process
Keq	equilibrium constant
Ko	M-M constant for O ₂ reduction process
Kp	M-M constant for P2 phosphorylation process
Kr	M-M constant for RuBP oxidation process
Ks	M-M constant for sucrose inhibition process
KPAR	PAR for half maximal CER
L-H	low to high [CO ₂]
L-L	low to low [CO ₂]
LAI	leaf area index
LIGHT	PAR generated electron-H ⁺ concentration
MC02e	final CO ₂ mass
MC02inj	injected CO ₂ mass

MC02o	initial CO ₂ mass
n	undefined number
NADP	nicotinamide adenine dinucleotide phosphate
NADPox	NADP oxidized state
NADPred	NADP reduced state
O ₂	oxygen
[O ₂]	chloroplastic (stromal) O ₂ concentration
P	inorganic phosphate
P1	chloroplastic P
P2	cytoplasmic P
PAR	photosynthetically active radiation
PCER	projected CER
PGA	phosphoglyceric acid
PGA1	chloroplastic PGA
PGA2	cytoplasmic PGA
PGly	phosphoglycolate
PNMAX	maximum CER-LIGHT response
R	universal gas constant
RC	respiration related coefficient
RD	dark respiration rate
Rm	membrane resistance to CO ₂ diffusion
RMP	ribose-5-phosphate
RuBP	ribulose-1,5-bisphosphate
RuBPc	RuBP carboxylase enzyme
RuBPc-o	RuBP carboxylase/oxygenase enzyme
RuBPo	RuBP oxygenase enzyme
RuP	ribulose-5-phosphate
SDBP	sedoheptulose-1,7-bisphosphate
SDMP	sedoheptulose-7-phosphate
SLW	specific leaf weight
SMP	sucrose phosphate
SPAR	soil-plant-air-research
STARCH	chloroplastic starch
SUCROSE	cytoplasmic sucrose
T	temperature
t	time
[TRANSPORT]	DHAP1 transport to DHAP2

UDP	uridine diphosphate
UDPG	uridine diphosphate glucose
UTP	uridine triphosphate
Vc	carboxylation reaction rate, 1 substrate
Vcmax	maximum Vc possible, 1 substrate
Vco	Vc with O ₂ competitive inhibition
Vcomax	maximum Vco possible
Vcr	carboxylation reaction rate, 2 substrates
Vcrmax	maximum Vcr possible
Vdpmax	maximum possible DHAP1-P2 [TRANSPORT]
Vdsmax	maximum possible SUCROSE formation rate
Vo	O ₂ reduction rate, 1 substrate
VOLUME	chamber volume
Vomax	maximum possible Vo, 1 substrate
Vor	O ₂ reduction reaction rate, 2 substrates
Vormax	maximum possible Vor, 2 substrates
XMP	xylulose-5-phosphate
[]	concentration
[1]	chloroplastic concentration
[2]	cytoplasmic concentration
[]	time rate of change
[]n	normalized values
Δ	change in
v	frequency
μ	micro
*	multiplier operand
**	power operand

APPENDIX 2
GLOSSARY OF TERMS

- allosteric.....enzyme activity affected by inhibitors or promoters
- autotrophic.....cells that can use CO_2 directly as their sole
source of carbon and light as their sole source
of energy
- carbohydrates.....compounds with the empirical formula $[\text{CH}_2\text{O}]_n$
and their derivatives; well known examples are
starch, sucrose, glucose, and cellulose
- carboxylation.....any reaction process in which CO_2 is reduced
- chlorophylls.....light trapping pigments of photosynthetic cells
- chloroplasts.....chlorophyll containing plastids suspended within
photosynthesizing cells
- cofactor.....additional nonprotein structures required for
activation of an enzyme
- cytoplasm.....the living component of cells in which organelles
are suspended
- dark respiration.....the controlled release and utilization of energy
from the oxidation of carbohydrates in metabolism
processes of growth and maintenance in living
tissues
- downhill.....reactions that have a negative standard free
energy change and will proceed spontaneously
under standard conditions; an exergonic process

- endergonic.....reactions that have a positive standard free energy change and will not proceed spontaneously in the direction written under standard conditions; an uphill process
- enzymes.....highly specialized class of protein molecules which catalyze virtually all metabolic reactions
- equilibrium.....state that exists when no changes take place in a system's properties over time
- exergonic.....reactions that have a negative standard free energy change and will proceed spontaneously in the direction written; a downhill process
- fluorescence.....the loss of energy originally absorbed by reemission of light quanta; the emitted light is of longer wavelength than the absorbed light's wavelength
- Gibb's free energy....a measure of a system's total energy changes relative to changes in entropy
- gross photosynthesis..total CO_2 taken up by the Calvin cycle carboxylation process
- glucose.....the most abundant simple sugar and the precursor of sucrose and starch; its chemical formula is $\text{C}_6\text{H}_{12}\text{O}_6$
- heterotrophic.....cells that cannot use CO_2 but must obtain carbon and energy from relatively complex reduced compounds such as sucrose
- in vitro.....procedures that occur outside the living cell; in an artificial environment

- in vivo.....procedures that occur within the living cell;
 in natural conditions
- organelles.....the highest level of intracellular organization;
 various supramolecular complexes such as nuclei,
 mitochondria and chloroplasts
- oxidation.....the chemical loss of electrons
- phloem.....system for transport from organ to organ and
 storage of photosynthetic products found in higher
 plants
- phosphorylation.....a chemical process in which a phosphate group is
 coupled to another compound; in biological systems
 phosphate groups relate to chemical energy status
- photorespiration.....the reduction of O_2 by RuBP; a process that
 directly competes with photosynthesis for available
 RuBP
- photosynthate.....the general products of photosynthesis such as
 starch and sucrose; carbohydrates produced by
 photosynthesis
- photosynthesis.....the chemical reduction of CO_2 with RuBP as a
 reducing agent, RuBP being the product of a
 biochemical cycle driven by light energy
- photosynthetically
 active radiation.....electromagnetic radiation with wavelengths in the
 range 400 to 700 nanometers; wavelengths that can
 be absorbed by photosynthetic pigments
- pool.....the available supply of a compound in the chloro-
 plastic reaction tank or the cytoplasmic reaction
 tank

- reduction.....the chemical gain of electrons
- reversible.....thermodynamically, a process that occurs without change in entropy or biochemically, a process that enzymatically and energetically can proceed in either direction
- standard conditions...conditions in which standard free energy change occurs for a reaction; 1.0 molar concentration for all reactants, pH equal to 7.0 and 25° C
- starch.....a carbohydrate characterized by long chains with chemical formula $(C_6H_{12}O_6)_n - H_2O$ where n is any number
- steady flow.....condition that occurs in an open system when flow out of the system equals flow into the system
- steady state.....condition that occurs when there are no net changes in a system's characteristics with time
- stoichiometry.....the mass relationships in chemical formulas and equations
- stroma.....the fluid component of a chloroplast; the chloroplastic site of the Calvin cycle and various other biochemical processes
- substrate.....components, reactants or precursors involved in a chemical reaction
- sucrose.....a carbohydrate with chemical formula, $C_{12}H_{22}O_{11}$; the primary photosynthate transported throughout the plant
- tank.....a term used synonymously with pool referring to the available supply of a compound

thylakoid.....the chloroplastic structure housing pigments where
the light reactions take place

trifoliates.....the three leaf structural units common to soybean
plants

uphill.....synonomous with energonic; an energy requiring
reactions under standard conditions

APPENDIX 3 UNITS LISTING

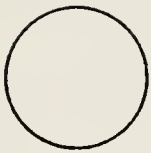
Length	m	meters
	cm	centimeters
	nm	nanometers
Area	m**2	meters squared
	dm**2	decimeters squared
	cm**2	centimeters squared
Volume	m**3	meters cubed
	l	liters
	μ l	microliters
Time	hr	hours
	min	minutes
	sec	seconds
Mass	kg	kilograms
	g	grams
	mg	milligrams
	mg chl	milligrams chlorophyll
	μ g	micrograms
	μ mol	micromoles
Concentration	kg/m**3	kilograms/meter cubed
	vpm	volumetric parts/million
	mM	millimolar
	μ M	micromolar
Pressure	mbar	millibars

APPENDIX 3 (contd.)

Flux	$\text{kg/m}^{**2}/\text{hr}$	kilograms/meter squared/hour
	$\text{mg/dm}^{**2}/\text{hr}$	milligrams/decimeter squared/hour
	$\mu\text{mol/mg chl/hr}$	micromoles/milligram chlorophyll/hour
	$\mu\text{E/m}^{**2}/\text{sec}$	microEinsteins/meter squared/seconds
Total Flux	E/m^{**2}	Einsteins/meter squared

Note: In the units notation the double asterisk (**) and the slash (/) are read "to the power of" and "per," respectively. All mole quantities refer to gram moles.

APPENDIX 4 SYMBOL LISTING



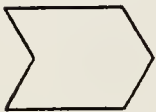
Source

mass or energy source outside of system; a forcing function



Tank

a compartment of mass or energy storage within the system storing a quantity as the balance of inflows and outflows; a state variable



Interaction

interactive intersection of two pathways coupled to produce an outflow in proportion to a function of both; limiting factor action



Interaction

Michaelis-Menten form



Pathway indicator

defines specific pathways

APPENDIX 5 PROBABLE ERROR ANALYSIS OF CO₂ MASS BALANCE MEASUREMENTS

The primary experimental result from the chamber control system was the carbon dioxide exchange rate (CER) or less precisely, the photosynthetic rate. To control chamber CO₂ concentration, the CO₂ injection rate was discretely adjusted to equal CER. Equation (56) is a quantitative statement of this mass balance control algorithm.

$$\text{CER} = (\Sigma \text{MCO}_{2\text{inj}} + \text{MCO}_{2\text{i}} - \text{MCO}_{2\text{e}}) / (0.415) (\text{AREA}) \quad (56)$$

A more explicit version of this equation must be more detailed:

$$\text{MCO}_{2\text{inj}} = \text{FLOW} * \text{TIME},$$

$$\text{MCO}_{2\text{i}} = [\text{CO}_2]_{\text{m}} * \text{VOLUME}, \text{ and}$$

$$\text{MCO}_{2\text{e}} = [\text{CO}_2]_{\text{m}} * \text{VOLUME}$$

where FLOW is the injection mass flow rate, TIME is the time that the injection solenoid is open, $[\text{CO}_2]_{\text{m}}$ is the infrared gas analyzer measurement of chamber CO₂ concentration and VOLUME is the chamber volume. An additional consideration not included at all in equation (56) is a correction for diffusion leakage (DL) made on a chamber by chamber basis. Expanding equation (56) yields

$$\text{CER} = \frac{(\text{FLOW} * \text{TIME}) + ([\Delta \text{CO}_2]_{\text{m}} * \text{VOLUME})}{(0.415) (\text{AREA})} + \text{DL} .$$

Functionally, this equation can be written

$$\text{CER} = f[\text{FLOW}, \text{TIME}, [\Delta \text{CO}_2]_{\text{m}}, \text{VOLUME}, \text{AREA}, \text{DL}].$$

Partial derivatives from this relationship are as follows:

$$\frac{\partial f}{\partial \text{FLOW}} = \text{TIME}/(0.415) (\text{AREA}),$$

$$\frac{\partial f}{\partial \text{TIME}} = \text{FLOW}/(0.415) (\text{AREA}),$$

$$\frac{\partial f}{\partial [\Delta \text{CO}_2]_m} = \text{VOLUME}/(0.415) (\text{AREA}),$$

$$\frac{\partial f}{\partial \text{VOLUME}} = [\Delta \text{CO}_2]_m/(0.415) (\text{AREA}),$$

$$\frac{\partial f}{\partial \text{AREA}} = -\text{FLOW} * \text{TIME}/(0.415) (\text{AREA})^2 \text{ and}$$

$$\frac{\partial f}{\partial \text{DL}} = 1.$$

Probable error as a fraction of CER is given by

$$\begin{aligned} \frac{\Delta \text{CER}}{\text{CER}} = \frac{1}{f} \left[\left(\frac{\partial f}{\partial \text{FLOW}} \Delta \text{FLOW} \right)^2 + \left(\frac{\partial f}{\partial \text{TIME}} \Delta \text{TIME} \right)^2 \right. \\ + \left(\frac{\partial f}{\partial [\Delta \text{CO}_2]_m} \Delta [\Delta \text{CO}_2]_m \right)^2 + \left(\frac{\partial f}{\partial \text{VOLUME}} \Delta \text{VOLUME} \right)^2 \\ \left. + \left(\frac{\partial f}{\partial \text{AREA}} \Delta \text{AREA} \right)^2 + \left(\frac{\partial f}{\partial \text{DL}} \Delta \text{DL} \right)^2 \right]^{1/2} \end{aligned}$$

To solve this equation the accuracies of each variable have been estimated either by using direct calibration, manufacturers calibration or best guess. Approximations of accuracies are as follows:

$\Delta \text{FLOW} = 9.4 * 10^{-8}$ kg/sec--this value is based on direct calibrations which consistently showed a secular drift in flow rate that was larger than the manufacturers calibrated accuracy,

$\Delta \text{TIME} = 3.2 * 10^{-7}$ sec--this value is based on the manufacturer's calibration of the computer's real time clock,

$\Delta [\Delta \text{CO}_2]_m = 7.8 * 10^{-6}$ kg/m**3--this value is based on the manufacturer's calibrated accuracy which had a larger uncertainty than was obtained in direct calibrations,

$\Delta \text{VOLUME} = 0.05$ m**3--this value is relatively large because of uncertainty over the exact volume of duct work in the control chamber; the value is a best guess,

$\Delta \text{AREA} = 0.0001$ m**2--this value is also a best guess conservatively assuming that length and width were known to within 1 cm,

$\Delta \text{DL} = 2.0 * 10^{-5}$ kg/m**2/hr--this value is a best guess of uncertainty in the graphical techniques used to measure the diffusion leakage rate.

Two calculations will be made for different cases. First, the case of continuous injection into a high $[\text{CO}_2]_d$ chamber with a large leakage rate will be considered. Values are

$$\text{FLOW} = 2.8 * 10^{-6} \text{ kg/sec,}$$

$$\text{TIME} = 300 \text{ sec,}$$

$$[\Delta \text{CO}_2]_m = 5.9 * 10^{-5} \text{ kg/m**3,}$$

$$\text{VOLUME} = 1.6 \text{ m**3,}$$

$$\text{AREA} = 1.0 \text{ m**2 and}$$

$$\text{DL} = -2.0 * 10^{-4} \text{ kg/m**2/hr.}$$

Using these values and the uncertainties outlined above probable error can be calculated. Individual terms are

$$\frac{\partial f}{\partial \text{FLOW}} = \Delta \text{ FLOW} = 6.8 * 10^{-5} \text{ kg/m**2/hr,}$$

$$\frac{\partial f}{\partial \text{TIME}} \Delta \text{TIME} = 1.3 * 10^{-12} \text{ kg/m}^{**2}/\text{hr},$$

$$\frac{\partial f}{\partial [\Delta \text{CO}_2]_m} \Delta [\Delta \text{CO}_2]_m = 3.0 * 10^{-5} \text{ kg/m}^{**2}/\text{hr},$$

$$\frac{\partial f}{\partial \text{VOLUME}} \Delta \text{VOLUME} = 7.1 * 10^{-6} \text{ kg/m}^{**2}/\text{hr},$$

$$\frac{\partial f}{\partial \text{AREA}} \Delta \text{AREA} = 2.0 * 10^{-7} \text{ kg/m}^{**2}/\text{hr},$$

$$\frac{\partial f}{\partial \text{DL}} \Delta \text{DL} = 2.0 * 10^{-5} \text{ kg/m}^{**2}/\text{hr}, \text{ and}$$

$$\text{CER} = 2.1 * 10^{-3} \text{ kg/m}^{**2}/\text{hr}, \text{ a typical measured rate.}$$

Substituting into the probable error equation the following ratio is obtained:

$$\frac{\Delta \text{CER}}{\text{CER}} = 0.037 = 3.7\%$$

Second, the case of a low $[\text{CO}_2]_d$ chamber with a typical injection time will be considered. For this case, CO_2 levels inside and outside the control chambers are nearly equal allowing DL to be dropped since no net diffusion occurs. Values are

$$\text{FLOW} = 2.8 * 10^{-6} \text{ kg/sec},$$

$$\text{TIME} = 50 \text{ sec},$$

$$[\Delta \text{CO}_2] = 2.5 * 10^{-5} \text{ kg/m}^{**3},$$

$$\text{VOLUME} = 1.6 \text{ m}^{**3},$$

$$\text{AREA} = 1.0 \text{ m}^{**2}.$$

Using these values the probable error can be calculated. Individual terms are

$$\frac{\partial f}{\partial \text{FLOW}} \Delta \text{FLOW} = 1.1 * 10^{-5} \text{ kg/m}^{**2}/\text{hr},$$

$$\frac{\partial f}{\partial \text{TIME}} \Delta \text{TIME} = 1.3 * 10^{-12} \text{ kg/m}^{**2}/\text{hr},$$

$$\frac{\partial f}{\partial [\Delta \text{CO}_2]_m} \Delta [\Delta \text{CO}_2]_m = 3.0 * 10^{-5} \text{ kg/m}^{**2}/\text{hr},$$

$$\frac{\partial f}{\partial \text{VOLUME}} \Delta \text{VOLUME} = 3.0 * 10^{-6} \text{ kg/m}^{**2}/\text{hr},$$

$$\frac{\partial f}{\partial \text{AREA}} \Delta \text{AREA} = 3.4 * 10^{-8} \text{ kg/m}^{**2}/\text{hr}, \text{ and}$$

CER = $4.3 * 10^{-4} \text{ kg/m}^{**2}/\text{hr}$, a typical measured rate.

Substituting into the probable error equation the following ratio is obtained:

$$\frac{\Delta \text{CER}}{\text{CER}} = 0.075 = 7.5\%.$$

Looking at the contributing terms, the FLOW term and the $[\Delta \text{CO}_2]$ term are important in both examples. In addition diffusion leakage in the high $[\text{CO}_2]$ case is important. Of these uncertainties diffusion leakage and the injection flow rate have the greatest room for improvement through careful experimental design. Even though uncertainty in $[\text{CO}_2]_m$ is based on the manufacturers calibration it too can be improved by dedicating a different gas analyzer to each CO_2 level allowing full scale to be reduced. Overall the probable error in CO_2 mass balance measurements is acceptable.

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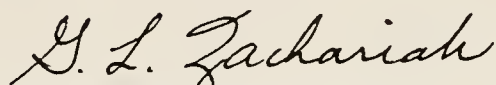
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BIOGRAPHICAL SKETCH

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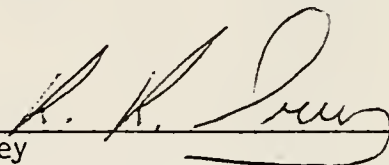
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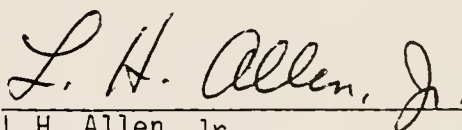
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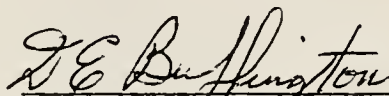
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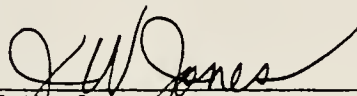
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This dissertation was submitted to the Graduate Faculty of the College of Engineering and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June 1981



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